Gene Drive and Sexual Selection in House Mice

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English Summary

At first sight, biological organisms appear as harmonious entities, armed with features exquisitely fine-tuned to its survival and reproduction. This is no accident: Darwin's theory of evolution by natural selection entails that genes spread through populations, exactly *because* they contribute to organismal fitness. However, biologists are uncovering ever more cases of genetic entities, so called drive genes, that are at odds with the notion of the organism as the (sole) fitness-maximising agent. By violating the Mendelian rules of inheritance, drive genes successfully spread through populations, often despite detrimental consequences for their carriers.

The t haplotype in house mice (*Mus musculus domesticus*) is the paradigm example of a drive element. This cluster of genes, occupying about one third of mouse chromosome 17, is fatal to the fitness of its hosts: t/t homozygotes die from recessive lethal mutations during embryogenesis. On its own, this would predict immediate extinction of the t haplotype. Yet, t haplotypes systematically manipulate gametogenesis in their favour. As a result $+/t$ heterozygous males transmit the t haplotype, instead of the usual 50%, to up to 90% of their progeny. This selfish disruption of the fair Mendelian ratios has allowed t haplotypes to spread through house mouse populations around the world in spite of the harm they incur to the individuals and populations harbouring them.

The stable presence of a drive gene has profound evolutionary consequences. Once the 'fair' rules of meiosis have failed at maintaining the integrity and function of the organism, we expect selection on the organism to evolve other measures to suppress drive element's selfish acts. For example, it has been suggested that females may avoid fertilisation by t haplotype carrying sperm, because such avoidance will protect her offspring form the t related fitness costs. Two mechanisms of sexual selection against drive genes have received particular attention in the literature. First, females could avoid t fertilisation by avoiding t-haplotype-carrying males prior to mating. Second, females could avoid t fertilisation by systematically mating with several males (termed polyandry). This second hypothesis is based on the premise that drive-carrying males

are compromised in their sperm competitive ability. In this thesis, I have investigated the joint evolution of a gene drive and the female mating behaviour. Using a variety of methodological approaches, ranging from theoretical modelling to laboratory experiments to data analysis from a natural population, I have addressed the following two questions.

(1) How does female mating behaviour affect the frequency dynamics of a drive gene? Understanding the evolutionary forces that determine the frequency of drive genes in natural populations is a long-standing focus of evolutionary biology. In the case of the *t* haplotype, naturally observed frequencies are typically much lower than expected based on drive and homozygote lethality alone (this discrepancy between the standard model and data has been termed 'the *t* frequency paradox'). In this dissertation, I show that the inclusion of female mating behaviour to the standard model, polyandry in particular, greatly improves our *t* frequency predictions and can largely explain the low *t* frequencies observed in nature. In a mate choice experiment, we show that females are indeed able to avoid fertilisation by drive-carrying males. The absence of clear social preferences during the choice test suggested that this fertilisation bias is largely driven by polyandry and subsequent sperm competitive effects. The importance of sperm competitive effects was corroborated in a second study. Here, we provide direct evidence that *t* carrying males are heavily compromised in their sperm competitive ability. Accordingly, *t*-carrying males only sired 19% of offspring when competing fertilisation with a wild-type male. We further show that this disadvantage has direct implications on population *t* frequencies. We found that in a selection experiment, where mice were kept under strictly monogamous or polyandrous conditions over the course of 20 generations, *t* frequencies have significantly decreased in the polyandrous selection lines, while remaining constant and high in the monogamous lines. For the first time in any drive system, we provide evidence that such sperm competitive effects are directly relevant under natural conditions. In an intensively monitored house mouse population outside Zürich, we found that the reproductive success of *t* males was particularly strongly affected by sperm competition. Moreover, females are highly polyandrous: over 47% of litters born during the 4.5 year observation period were sired by more than one father. In line with the 'polyandry hypothesis', we observe a decline in population *t* frequencies during the investigation period.

(2) How does the presence of drive gene affect the evolution of female mating behaviour? Understanding the evolutionary forces that drive the evolution of female mating behaviour (such as mate choice and polyandry) is a highly debated topic in

evolutionary research. It has been hypothesised that mate choice and/or polyandry is beneficial to females because they will result in fertilisation by males of a high genetic quality ('good genes' or 'good sperm' hypotheses). Yet conventional genetic mechanisms are usually insufficient to maintain variation in male genetic quality, thereby rendering any form of choice obsolete (this problem is generally known as the 'lek paradox'). Using a theoretical model, we show here that the presence of a drive gene can greatly facilitate the evolution of female choice, even in circumstances where such a choice is associated with direct fitness costs. First, costly drive-male avoidance is beneficial to females because it helps them avoid drive related fitness costs. Second, costly drive-male avoidance is evolutionarily stable, because gene drive maintains variation in male genetic quality at equilibrium. As a result, the lek paradox is largely avoided. Despite this compelling theoretical argument, we have found little evidence that the presence of the *t* haplotype has triggered the evolution of female drive avoidance in the circumstances considered here. While polyandry helped females avoid *t* related litter losses in the laboratory, we find no signs of selection on polyandry rates under natural conditions. Moreover, we find little evidence that polyandry is heritable. Thus, even in the case of drive-triggered selection on polyandry, it is unlikely that the trait would respond to selection.

Deutsche Zusammenfassung

Seit jeher faszinieren uns biologische Organismen als harmonische, perfekt an ihre Umgebung angepasste Einheiten. Aus evolutionsbiologischer Sicht ist diese Harmonie nicht überraschend: Darwin's Theorie der natürlichen Auslese postuliert, dass nur die Gene in einer Population erhalten bleiben, die zum erfolgreichen Überleben und zur Fortpflanzung des Individuums beitragen. Biologen finden allerdings immer häufiger Genabschnitte, die diesem Prinzip der Individualselektion widersprechen. Durch das Aushebeln der Mendelschen Erbregeln breiten sich diese sogenannten 'egoistischen Gene' erfolgreich in Populationen aus, obwohl sie für ihre Träger schädlich sind.

Der *t* Haplotyp in Hausmäusen (*Mus musculus domesticus*) ist ein klassisches Beispiel eines 'egoistischen Genes'. Diese spezielle Form des Chormosoms 17 in Hausmäusen hat fatale Folgen für seine Träger. Aufgrund von Letalmutationen stirbt jede Maus, die zwei Kopien dieses Genes trägt, noch im Mutterleib. Trotz dieses tödlichen Effektes hat sich der *t* Haplotyp Mauspopulationen auf der ganzen Welt unterwandert. Dieser offenbare Widerspruch zum Prinzip der Individualselektion erklärt sich dadurch, dass der *t* Haplotyp die Mendelschen Erbfrequenzen zu seinen Gunsten manipuliert. Die Mendelschen Gesetze besagen, dass alle Gene in einem Organismus dieselbe, 'faire' 50% Chance auf eine Weitervererbung an die nächste Generation haben. Dies ist nicht der Fall hier: anstelle des üblichen 1:1 Verhältnisses übertragen Männchen den *t* Haplotypen an bis zu 90% ihrer Nachkommen. Diese Segregationsverzerrung verschafft dem t Haplotypen einen 'unlauteren' Vorteil und erlaubt es ihm, sich im 'Wettlauf der Gene' auf Kosten aller anderen Gene (und des Individuums als Ganzes) durchzusetzen.

Die Präsenz von egoistischen Genen hat weitreichende evolutionäre Konsequenzen. Man würde erwarten, dass die betroffenen Organismen Strategien entwickeln, die die Weiterverbreitung dieses genetischen Parasiten in Schach zu halten. So könnten Weibchen beispielsweise ihren Nachwuchs vor den tödlichen Folgen des *t* Haplotypen schützen, indem sie die Befruchtung durch *t* Haplotyp-tragende Männchen verhindern. Zwei Mechanismen der sexuellen Selektion gegen egoistische Gene sind in der Literatur diskutiert worden. Zum einen könnten Weibchen eine Befruchtung durch

t Männchen vermeiden, indem sie sich während der Partnerwahl häufiger für Männchen entscheiden, die keinen *t* Haplotypen tragen (sogenannte Wildtyp-Männchen). Zum anderen könnten Weibchen die Befruchtung durch *t* Spermien vermeiden, indem sie sich systematisch mit mehreren Männchen verpaaren (Polyandrie). Diese zweite Hypothese beruht auf der Annahme, dass sich egoistische Gene wie der *t* Haplotyp negativ auf die Spermienkompetenz der Männchen auswirken. Im Rahmen meiner Dissertation habe ich das evolutionäre Zusammenspiel zwischen egoistischen Genen und diesen beiden Formen von sexueller Selektion genauer durchleuchtet. Mithilfe von theoretischen Modellen, Laborexperimenten an wilden Hausmäusen und Daten aus einer Wildpopulation habe ich zwei Fragen untersucht.

(1) Wie wirkt sich das weibliche Paarungsverhalten auf die Ausbreitung von egoistischen Genen aus? Seit mehr als einem halben Jahrhundert versuchen Evolutionsbiologen die Kräfte zu verstehen, die die Frequenz von egoistischen Genen in wildlebenden Populationen bestimmen. Gerade im Fall des *t* Haplotyps geben die in natürlichen Populationen beobachteten Frequenzen ein besonderes Rätsel auf. Diese sind nämlich drastisch tiefer als man aufgrund der Segregationsverzerrung erwarten würde (dieses Problem ist in der Literatur als '*t* Paradox' bekannt). In meiner Doktorarbeit konnte ich mit einer Reihe von Laborexperimenten und Daten aus einer wilden Population aufzeigen, dass weibliches Paarungsverhalten, und Polyandrie im Besonderen, diese Diskrepanz zwischen beobachteten und vorhergesagten Frequenzen grösstenteils erklären kann. In einem Partnerwahl-Experiment im Labor zeigen wir auf, dass Weibchen in der Tat die Befruchtung durch *t* Männchen verhindern können. Die Daten lassen vermuten, dass dieser Effekt vor allem auf Polyandrie und Spermienkonkurrenz, und nicht auf Partnerwahl zurückzuführen ist. Diese Schlussfolgerung konnten wir in einem zweiten Experiment weiter untermauern. Hier zeigen wir durch direkte Messungen, dass *t* Männchen bei der Spermienkonkurrenz besonders schlecht abschneiden. Dieser Nachteil hat unmittelbare Implikationen für die *t* Haplotyp Frequenz in Populationen. In einem Selektionsexperiments, in welchem Mäuse für 20 Generationen unter strikte monogamen oder polyandrischen Bedingungen gehalten worden sind, haben wir in den polyandrischen Selektionslinien einen signifikanten Rückgang der *t* Frequenz beobachtet. Im Gegensatz dazu sind in den monagamen Selektionlinien die *t* Frequenzen unverändert hoch geblieben. Die Resultate dieser beiden Laborstudien konnten wir mit Beobachtungen in einer wilden Population in der Nähe von Zürich weiter bestätigen. Auch hier scheint sich Spermienkonkurrenz besonders negativ auf den Fortpflanzungserfolg von *t* Männchen auszuwirken. Wir zeigen zudem,

dass Polyandrie in weiblichen Hausmäusen weit verbreitet ist. Im Schnitt haben 47% der Würfe mehr als einen Vater. Vor diesem Hintergrund ist es nicht überraschend, dass wir auch hier einen Rückgang des *t* Haplotypen über einen Zeitraum von 4.5 Jahren beobachten.

(2) Können egoistische Gene zu unserem Verständnis von weiblichem Paarungsverhalten beitragen? Biologen verstehen nach wie vor nur teilweise, warum sich Weibchen in vielen Arten mit mehreren Männchen verpaaren (Polyandrie). Auch die evolutionären Gründe für weibliche Partnerwahl sind äusserst umstritten. Eine vielbeachtete Hypothese besagt, dass Weibchen durch Polyandrie und/oder Partnerwahl sicherstellen können, dass ihr Nachwuchs von den Männchen mit der höchsten genetischen Qualität gezeugt werden. Diese Argumentationslinie führt allerdings zu einem logischen Problem (auch als 'Lek Paradox' bekannt). Eine Partnerwahl nach den Kriterien der genetischen Qualität macht nämlich nur dann Sinn, wenn sich die Männchen auch in ihrer Qualität *unterscheiden*. *Gerade wegen* der Partnerwahl würde man jedoch erwarten, dass sich *nur* die Männchen mit der besten genetischen Qualität fortpflanzen können und damit in der Population erhalten bleiben. In diesem Fall erübrigt sich jede Wahl. Mit einem theoretischen Modell können wir hier aufzeigen, dass dieser offenbare Widerspruch im Kontext von egoistischen Genen nur eine untergeordnete Rolle spielt. Dank der Segregationsverzerrung bleiben die nicht-präferierte Männchen nämlich in der Population vorhanden, obwohl sie nicht oder nur selten von Weibchen gewählt werden. Unser Modell sagt also vorher, dass Präsenz von egoistischen Genen die Evolution von weiblicher Partnerwahl und Polyandrie erleichtert. Trotz dieser deutlichen Modellvorhersage finden wir nur wenige Hinweise, dass der *t* Haplotyp in unserem Fall die Evolution von weiblichem Paarungsverhalten beeinflusst hat. Zwar können wir unter Laborbedingungen zeigen, dass Weibchen mithilfe von Polyandrie ihren Nachwuchs vor den tödlichen Folgen des *t* Hapltyps schützen können. Allerdings finden wir in unserer Wildpopulation keine Anzeichen dafür, dass Polyandrie den Reproduktionserfolg der weiblichen Mäuse beeinflusst. Wir zeigen zudem, dass das Auftreten von Polyandrie in der Wildpopulation vor allem von extrinsischen Faktoren wie der Häufigkeit von Geschlechtspartnern abhängt. Wir finden wenige Hinweise für eine intrinsische, genetisch oder individuell bedingte Prädisposition für das Verhalten. Vor diesem Hintergrund erscheint die Evolution von Polyandrie als Reaktion auf den *t* Haplotypen unwahrscheinlich.

Contents

Chapter 1

General Introduction

Life on earth is organised in hierarchically nested units. Genes assemble in chromosomes, chromosomes in genomes, genomes in cells, cells in organisms, organisms in kin groups, kin groups in colonies, colonies in species, species in entire ecosystems. Within this hierarchy, evolutionary biologists have traditionally thought —and often still think— of the organism as the unit at which natural selection occurs. This is no accident. More often than not, organisms appear as harmonious, integrated wholes, armed with features exquisitely fine-tuned to promote the survival and reproduction of the organism. Yet evolutionary research over the past 50 years has convincingly demonstrated that the traditional, organism-centred view of evolution is an illusion.

The harmony of the organism is an illusion from a *conceptual* perspective, because the fundamental logic of natural selection is not exclusive to organisms. Darwinian evolution by natural selection is an abstract concept. *Any* entity that (1) varies in populations, (2) replicates at different rates because of this variation and (3) leaves offspring that resembles itself (Lewontin, 1970), will cause systematic changes in population composition over time, and thus be subject to the Darwinian process of natural selection. Over the years, there have been fierce debates as to which of the above mentioned hierarchical levels are deserving of the label 'unit of selection' (Okasha, 2006; Williams, 1966; Nowak et al., 2010). Yet it seems clear that the three Darwinian principles of variation, inheritance and differential reproduction are met in at least some units other than the organism, particularly at the sub-organismal level (e.g. genes and cells). It follows that selection can simultaneously occur at multiple levels in the hierarchy, a phenomenon that is commonly termed *multilevel selection*. Importantly, once we see genes and cells as fitness maximizing agents in their own right, organisms can no longer be viewed as harmonious units, but rather as *collective* entities that result from a most extensive and elaborate collaboration among its constituent, lower-level parts. Because we expect lower-level parts to be primarily in search of their *own* Darwinian posteriority, cooperation among them requires an explanation, just as cooperation among organisms (Haig, 1997). Likewise, if selection can operate at multiple levels in the hierarchy, units that thrive on one level may be harmful at another. In cases where selection acts in opposing directions at different levels, *multilevel conflict* arises (Okasha, 2006; Keller, 1999).

The harmony of the organism is an illusion from an *empirical* perspective, because organisms regularly exhibit features that are of no apparent benefit to them individually. Altruism, where an organism pays a fitness cost to increase the fitness of another individual, is a classical example of a feature that does not benefit the organism itself.

Cancer, where cells within a body start to proliferate to the detriment of the organism as a whole, is another textbook example. All these phenomena have in common that they are at odds with the notion of organisms as (the sole) fitness maximizing agents. Although there are alternative explanations for the seemingly non-adaptive features (for example stochastic effects such as genetic drift), it has often been argued that these phenomena are the result of adaptive processes at a level other than the organismal. Accordingly, multilevel selection theory has played, and continues to play an important role in evolutionary biology, and has been applied to a wide range of biological phenomena. Famously, Hamilton (1964) used a gene level selection argument to explain altruistic behaviour among organisms.

The harmony of the organism is an illusion from the perspective of *natural history*, because the nested hierarchy of the living world was not a given from the start, but is itself an evolved property. It follows that organisms must have evolved by selective processes at *some* lower level. Higher levels in the hierarchy are thought to have formed in a series of events called major evolutionary transitions (Maynard Smith and Szathmary, 1995). These transition mark extraordinary points in evolutionary time where individually replicating entities give up their independence for the sake of a new, cooperating collective. These collectives eventually form a new, higher-level of biological organisation. Transitions have typically opened up fundamentally new avenues of evolutionary possibility. The success of modern multicellular life bears impressive testimony to what collectives can achieve if they work together. Fittingly, cooperation has been described as the main constructive force in the history of life on earth (Nowak, 2006; Maynard Smith and Szathmary, 1995). Yet there is a flip side: collaboration typically renders collectives susceptible to exploitation from within. The formation of a new collective hence requires rigid measures to suppress internal conflict. In other words, it is conflict among lower-level particles that has probably driven them into the formation of a new, higher-level collective. Yet, newly formed collectives can only be functional as new units if they manage to overcome the very forces that have given rise to it in the first place: conflict among its parts.

If the illusion of the harmony of the organism is such a convincing one to us today, it is because modern multicellular organisms have evolved an entire suite of fascinating adaptations that control and suppress internal conflict. Previous research suggests that integral features of complex organisms such as recombination, the uniparental inheritance of mitochondrial genomes, the inactivation of DNA in gametes, to only name a few, can be seen as collective, organismal measures to suppress conflicts among sub-

organismal units. The suppression of internal conflict entails that the adaptive process acts predominantly *between* and not *within* individuals. It is thus not surprising that, to the present-day observer, the biological world looks to be dominated by features that benefit the organism. Yet, there are instances when the internal control mechanisms break down, thereby exposing lingering sub-organismal conflicts that have principally been settled millions of years ago. Such conflicts remind us of the burdens that had to be overcome when complex multicellular life evolved in the first place.

1.1 Gene Drive: Multilevel Conflict in Action

The present thesis investigates a type of multilevel conflict that has, perhaps like no other, challenged our concept of harmony within the organism. It deals with selection at two levels, the level of the gene/allele (genetic selection) and the level of the diploid organism (organismal selection). An essential adaptation of diploid organisms to prevent internal conflict is the Mendelian 50:50 rule of chromosomal segregation during meiosis. Over the past last century, biologists have found ever more examples of genetic entities called drive genes that do not obey the Mendelian rules of fair play. Instead, they manipulate gamete production in their own favour. As a result, they are transmitted to systematically more than 50% of the offspring. This phenomenon has been called gene drive, meiotic drive, segregation distortion, or transmission ratio distortion (throughout this thesis, I will be using these terms interchangeably).

The deviation from Mendelian segregation has profound evolutionary implications. A great framework to conceptualize what drive genes do and why they result in multilevel conflict is the Price equation. George Price himself already recognized the natural applicability of his framework to situations where selection acts at multiple levels (Price, 1972; Frank, 1995). For example, the Price formalism has played an important role in the development and popularisation of kin selection theory (see Frank (1995) for a review). To develop a clear understanding of the conflict considered throughout this thesis, let us apply Price formalism to diploid genetics by considering an abstract example of a drive gene (adopted and modified from (Okasha, 2006) and (McElreath and Boyd, 2008)).

An Abstract Example of a Drive Gene

Let us consider a population of n diploid organisms, where two alleles D and d segregate at a given locus. As mentioned above, we investigate selection at two levels: (haploid) genes and (diploid) organisms. We thus think of alleles as the lower-level, particle unit (D and d), and of the diploid organisms ($D/D, D/d, d/d$) as the higher-level, collective unit. Because we are dealing with diploid organisms, collectives (organisms) will always consist of groups of *two* particles (alleles). According to the hierarchical structure (allele, individual, population), we can count the frequency of D alleles at three levels: p_{ij} counts the frequency of D alleles in the i-th allele of the j-th organism. This frequency can either be 0, if allele i is d, or 1, if allele i is D. Likewise, p_j counts the frequency of D alleles in organism j, where $p_j = \frac{1}{2}\sum_{i=1}^{2} p_{ij}$. Hence, p_j can have values 0, 0.5, or 1 depending on whether the organism genotype is d/d, D/d, or D/D, respectively. Finally, we can describe the frequency of D alleles in the entire population by $p = \frac{1}{n}\sum_{j=1}^{n} p_i = \frac{1}{2n}\sum_{j=1}^{n}\sum_{i=1}^{2} p_{ij}$.

In order to find out how the frequency of allele D will change over evolutionary time, we need to know how successful our two selective units, alleles and organisms, are in terms of offspring production (fitness). Let us thus define w_{ij} as the absolute number of copies that allele ij bequeaths to the gamete pool. It is a measure of how well an allele does *within* a particular organism. Likewise, we define w_j as the number of offspring produced by organism j. It thus measures how well groups of alleles fare as a *collective*, that is in competition *between* organisms. Alternatively, and equivalently, we can think of the two hierarchical selection levels as temporarily separated, with w_{ij} measuring selection at the haploid/gamete stage, whereas w_j quantifies selection in the diploid phase of an organism's life cycle. Price has derived a general framework that allows us to calculate the expected allele frequency change per generation in the population Δp (i.e. the amount of evolutionary change) as a function of selection at two levels. We have

$$\bar{w}\Delta p = \underbrace{Cov(w_j, p_j)}_{\text{organismal selection}} + \underbrace{E[Cov(w_{ij}, p_{ij})]}_{\text{genetic selection}}. \tag{1.1}$$

This equation tells us that our quantity of interest, the overall evolutionary change Δp, can be expressed as the sum of two terms. The first term, $Cov(w_j, p_j)$, denotes the covariance of the D allele number in an organism and the fitness of the organism *as a whole*. It thus measures how an organisms D-allele 'dosage' is associated with the

productivity of the collective, thus quantifies the intensity of selection at the organismal level (between organisms). The second term, $E[Cov(w_{ij}, p_{ij})]$, calculates the average covariance of allelic values with its fitness *within* a collective (organism). It quantifies the intensity of selection at the genetic level (within organisms). Finally, the absolute evolutionary change is weighed by the average fitness of the entire population $\bar{w}$. This formulation offers a great toolkit to appreciate the importance of Mendelian inheritance for the integrity of the organism, as well as to see what happens when the Mendelian rules are violated (as is the case with gene drive).

Mendel's invisible hand The two-level Price equation helps us to see why the fair rules of Mendelian inheritance during meiosis play such an essential role in aligning the interest of the individual particle with the interest of the organism as a whole. Let us assume that allele D does not contribute to the collective (see second column in schematic Fig. 1.1), in other words, it has detrimental effects on organismal fitness. Because a higher 'dosage' of D alleles will decrease an organism's reproductive output, the first covariance measuring selection between organisms will be negative. Importantly, if segregation ratios are perfectly Mendelian, the probability of transmission will, by definition, be the *same* for all alleles. In homozygous individuals D/D and d/d, a non-zero covariance between p_{ij} and w_{ij} is not possible, since there is no variance in either term. In heterozygote organisms D/d, there is variation in p_{ij} but not in w_{ij}, as both alleles have identical chances of transmission. Hence, *meiosis eliminates all selection within organisms*, and we have $E[Cov(w_{ij}, p_{ij}] = 0$. The overall evolutionary change will thus *solely* depend on the strength and direction of organismal selection. Because the organismal selection term $Cov(w_j, p_j)$ is negative, selection will eventually remove detrimental allele D from the population (as shown in Fig. 1.1).

Thus, with Mendelian segregation, natural selection will act, as if guided by an invisible hand, in the direction that optimizes the fitness of collective, i.e. the organism as a whole. Because selection within a genotype is eliminated, only alleles that contribute to the fitness of the collective (organism) can spread or be maintained in a population (in our example, allele d). We can now fully appreciate why under specific conditions (Mendelian inheritance), the traditional assumption of organisms as unbreakable, harmonious entities is perfectly justified.

Breaking Mendel's laws The importance of the rules of meiosis for the integrity of the organism are best seen when violated. Under the regime of Mendelian segregation, there is no possibility for opposing selection at the two levels because selection is *exclusive* to the level of the organism. This changes dramatically once we add gene

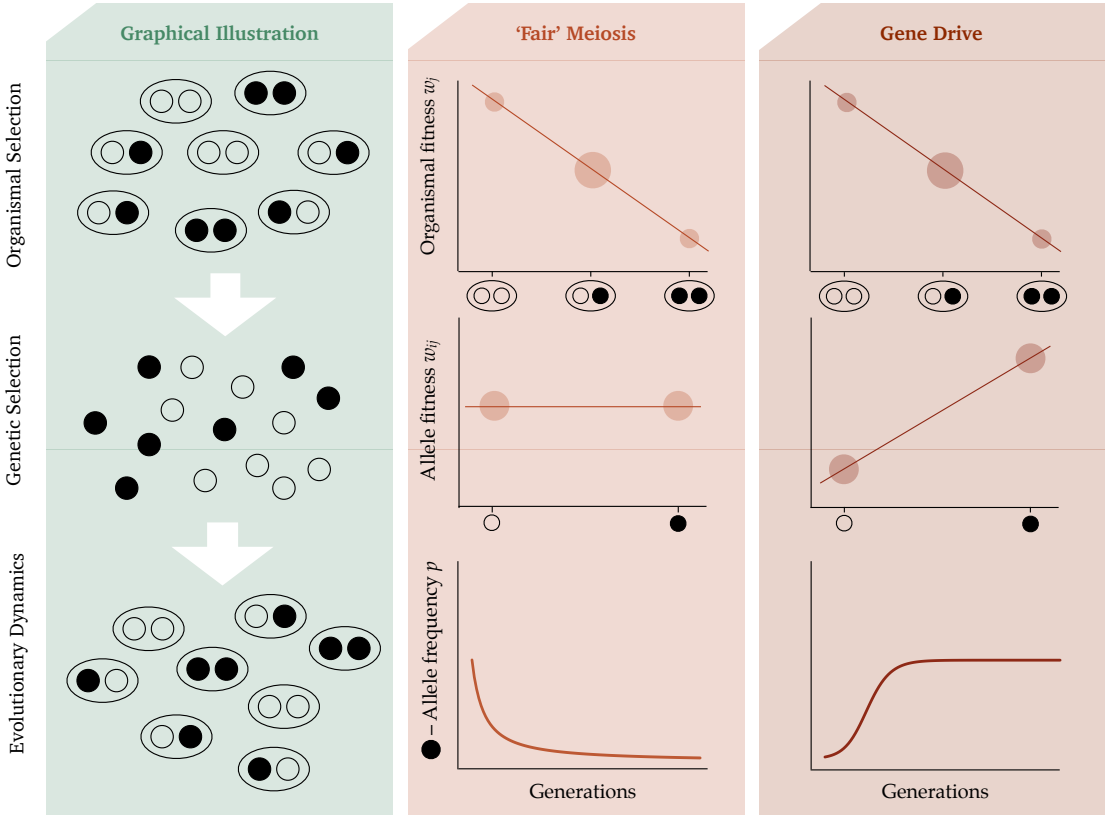

Figure 1.1. Schematic representation of the multilevel conflict considered throughout the thesis. Black-filled and open circles represent two types of alleles. Groups of two alleles cluster in a diploid organism. Selection can occur at two hierarchical levels: *between* organisms (organismal selection, top row) and among alleles *within* an organism (genetic selection, centre row). Let black alleles be detrimental to organismal fitness. In a scenario with Mendelian segregation (centre column), selection occurs exclusively at the level of the organism. As a result, the harmful black allele is removed from the population by negative organismal selection (bottom centre). In a scenario where black allele shows gene drive (right column), selection acts in opposing directions at the two levels (multilevel conflict). As a result, the harmful black allele is stably maintained in the population at the point where the two selective forces are in balance (bottom right).

drive to the equation. Drive genes systematically bias the fair 50:50 transmission ratios of Mendelian inheritance in their *own* favour. In our multilevel Price equation, the intensity of gene drive (equivalent to genetic selection) is measured by the second term in the equation, $E[Cov(w_{ij}, p_{ij}]$. Depending on the sign of the two selection terms, we expect a different evolutionary outcome. Suppose that allele D distorts segregation ratio in heterozygotes in its favour ($E[Cov(w_{ij}, p_{ij}] > 0$). If allele D is selectively neutral or positively selected at the organismal level, selective forces at both levels will act in the same direction, resulting in a positive overall evolutionary change. As a result, allele D will spread to fixation. It is unknown how frequently such drive related selective sweeps occur in nature, because —once fixed in a population— we expect drive genes to lose their driving ability through the accumulation of deleterious mutations. At this point, the presence of a past driver can no longer be detected.

Probably the most interesting scenario occurs when allele D is detrimental to the collective fitness (as shown in schematic Figure 1.1). In this case, we have $Cov(w_j, p_j) < 0$. Now, the two selection terms have opposing signs, the hallmark of a multilevel conflict. The evolutionary outcome will now be a matter of the relative strength of the two opposing forces. If both are equally strong ($Cov(w_j, p_j) = -E[Cov(w_{ij}, p_{ij}]$) at some intermediate frequency p, there will no longer be evolutionary change ($\Delta p = 0$) and we have a polymorphic equilibrium (see right column in Fig. 1.1). We now have a situation where driver D stably persists in the population despite the harm it causes to the collective, certainly bad news for the organism and populations that harbour it.

Another benefit of the Price formalism is that it neatly highlights the conceptual connection between different types of multilevel conflict. We have seen that the presence of gene drive D begets a situation where competition among particles results in suboptimal outcomes for the collective as a whole. This tension between individual and collective interest is common to all forms of multilevel conflict. In the case of the widely discussed problem of cooperation among organisms, for example, a group of cooperating individuals will typically fare well at the collective level ($Cov(w_j, p_j) < 0$, where p_j now denotes the proportion of helper individuals in a group). Yet such altruistic behaviour is readily exploited by free-riders *within* groups ($E[Cov(w_{ij}, p_{ij}] < 0$). Hence, all problems of multilevel conflict are formally equivalent to social conflicts.

Real-World Examples of Drive Genes

Drive genes are not (only) interesting inventions of evolutionary theorists, but they exist in the real world. Over the past century, and recently facilitated by the genomic revolution, biologist's have discovered countless examples of genetic entities that exactly exhibit the properties discussed above (Burt and Trivers, 2006).

Drive is costly While successful at the gamete level, drive systems typically impose serious costs on the individuals that carry them (Burt and Trivers, 2006). Half of the gametes are commonly destroyed, imposing a fertility cost. Many known drive systems comprise entire gene clusters that are transmitted as a unit because they suppress recombination. Such suppression of recombination often results in the accumulation of deleterious mutations. When drive occurs on a sex chromosome, the individual will produce offspring of only one sex. As sex chromosome drive also distorts sex ratios, drive individuals will produce more offspring of the commoner sex, reducing the fitness of the organism. As we have seen in our conceptual example above, it is this cost imposed on the organism (and, likewise, on the other genes in the genomes) that provokes multilevel conflict. Only if the drive gene imposes a cost to the collective, the organism, selection acts in opposing directions at different levels.

Drive is widespread There is increasing evidence to suggest that drive systems are not isolated freak accidents, but a widespread phenomenon across diploid life (Burt and Trivers, 2006). Whenever a diploid organism produces haploid gametes, there is potential for drive. Naturally occurring drive systems have been described in plants, fungi, insects, mammals, and birds, and these discoveries tend to occur in the best studied organisms (Burt and Trivers, 2006). For example, drive systems have been found in all three main genetic model organisms (mice, *Drosophila*, and *C. elegans*). However, drive systems are inherently difficult to detect, requiring in depth cytological or genomic work over multiple generations. As a result, there are likely to be large numbers of drive systems that are currently not detected. New genomic techniques are opening new avenues for detecting drive, and it is likely that many more drivers will be discovered as genomic data becomes available for large pedigrees in increasing numbers of species (see for example Knief (2015) for a recently detected distorter in zebra finches thanks to large-scale genomic data).

One Conflict, Different Perspectives

So far, we have framed the evolutionary conflict triggered by drive genes as a 'vertical' conflict between alleles and diploid organisms. It is worth noting that partitioning selection into a *within* and *between* genotype component is only one way of looking at the conflict. There are alternative views. Accordingly, the question about the 'true' level of selection has led to fierce debate and considerable confusion over the years (levels of selection debate, e.g. Williams (1966), Wynne-Edwards (1962), but also see Nowak et al. (2010) and replies for a recent example). Despite this confusion, many authors see these allegedly rival views as mathematically equivalent ways of looking at the same phenomenon (McElreath and Boyd, 2008). Others have argued that they are at least complementary, with their use depending on the specific question in hand (Okasha, 2006). To put it in the words of McElreath and Boyd (2008): "There is only one world out there. It would be bad if changing the way we did the accounting of genes changed the answer."

The gene-level perspective According to the gene selectionist, selection ultimately only occurs at the level of the genes (Burt and Trivers, 2006; Haig, 1997; Dawkins, 1976; Williams, 1966). Organisms are viewed as mere vehicles, sophisticated survival machines to carry the true replicators, the genes, into the subsequent generation. Drive genes fit well with this view: the type of ruthless selfishness expressed by a drive gene is precisely what one would expect if genes were the true unit of selection. Accordingly, the conflict elicited by drive genes has often been characterised as a 'horizontal' conflict among the genes inside the genome. Three conflict parties or 'interest groups' are typically distinguished. The perpetrators of conflict, i.e. the drive genes and all genes on the drive-linkage group, will all profit from the driver's systematic transmission advantage. Alternative alleles at the same position (the drive locus), and all genes linked to them, will be excluded from the offspring, hence reducing their success. Genes at different, unlinked positions (i.e. the 'rest of the genome') all suffer from the fitness cost that the driver imposes on the organism as a whole, but they do not benefit from its transmission advantage. Because all conflict parties will defend their own interest, we end up with a situation of *(intra-)genomic conflict*. This gene's eye view on the conflict is insightful, and offers insights that were not apparent under the multilevel formalism. For example, the genomic conflict framework can help us see why recombination has been proposed as an adaptation to suppress genetic conflict. By disrupting possible associations between neighbouring genes, recombina-

tion may prevent the build-up of gene alliances that could potentially rise up against the collective interest (Leigh, 1971). Moreover, it has also been argued that successful drivers are rare because they will always be vastly outnumbered by the opposing conflict party (i.e. the rest of the genome) in terms of gene numbers. As a result of this numeric imbalance, the party defending the interest of the organism will always be likely to hold the upper hand.

The organism-level perspective In most scenarios of multilevel selection, selection at the lower hierarchical level appears as a transmission bias at the higher level (Michod, 1999; Frank, 1998). In the original, one-level version of the Price equation, the second term (which here measured within-genotype selection) is typically taken to denote 'transmission fidelity' of the character of interest. Traditionally, this term has been used to describe imperfect transmission of a particular character value from one generation to the next (for example through mutations). Similarly, drive will result in a transmission bias in the collective character (allele frequency within an organism p_j): a heterozygous individual with character value $p_j = 0.5$ will give rise to offspring that systematically deviate from this value. Accordingly, we may argue that selection is acting entirely on organisms, and all that drive does is systematically reduce the transmission fidelity of our focal, organismal character.

The Drive System Considered in this Thesis: The *t* Haplotype in House Mice

In this thesis, we investigate the evolutionary consequences of one of *the* textbook examples of a drive system: the *t* haplotype in house mice (*Mus musculus domesticus*). This genetic entity was accidentally discovered by two Russian scientists in 1927 (Dobrovolskaia-Zavadskaia and Kobozieff, 1927) and has not ceased to fascinate evolutionary biologists ever since. Two properties make the *t* haplotype a paradigm drive system.

Firstly, *t* haplotypes are subjected to positive selection within genotypes (gene drive). The *t* haplotype manipulates spermatogenesis in males. Instead of the normal 50%, heterozygote $+/t$ males transmit *t* sperm to 90% of their progeny (henceforth described with parameter τ, Lindholm et al. (2013); Klein et al. (1984)). In females, on the other hand, inheritance ratios are perfectly normal (Mendelian). Secondly, *t* haplotypes have dramatic fitness costs to the organism as a whole. In most cases, t/t homozygotes perish during embryogenesis due to recessive lethal mutations (homozy-

gote lethality).

The evolutionary conflict that directly follows from these two properties is substantial. If t haplotypes occur in a randomly mating population at frequency p_t, no less than $p_t^2\tau$ of all offspring will perish before they are born. If half of the population carry the t and $\tau = 0.9$, more than one in ten pups will die due to t/t lethal effects! This is a severe cost to individuals and populations who harbour the t. Yet, the t haplotype will stably persist in populations due to its systematic transmission advantage at the gamete level.

Structure and natural history The t haplotype comprises a whole cluster of linked genes that spans about 30 Mb on the proximal third of mouse chromosome 17 (Silver, 1993). This is equivalent to about 1.2% of the mouse genome. Based on the mouse reference genome alone, we expect more than 800 genes on the t linkage group. Four non-overlapping inversions block recombination and ensure that the gene complex transmitted as one undisrupted unit (Silver, 1993). This is important, as the t's drive effect is achieved by several genes on the t haplotype cluster (see below). Several t haplotype variants have been described, each carrying at least one recessive lethal mutation. Homozygotes for different, complementing t haplotype variants usually survive, but result in male sterility (Klein 1984). Based on the complementarity, at least 16 different t haplotypes have been described (Klein et al., 1984). The haplotype is thought to have arisen 1.5 to 3 million years ago (Hammer and Silver, 1993), and has been identified in all four house mouse subspecies. The striking similarity of t haplotypes among the different subspecies suggest a recent selective sweep of a particular t haplotype variant through all four subspecies (Hammer and Silver, 1993).

Molecular mechanism of drive Efforts to understand the molecular mechanism of drive have been relatively successful. Heterozygote males produce + and t sperm at equal proportions. Meiosis thus works perfectly normally, making the commonly used term 'meiotic drive' misleading in the context of the t haplotype. The distortion of Mendelian frequencies occurs after meiosis during spermatogenesis. A set of distorter loci disrupt flagellar function across all sperm (+ and t). At a later stage during sperm development, a t haplotype specific responder gene recovers sperm function in t sperm only. As a result of this nasty 'poison-antidote system', + sperm are heavily compromised in their swimming ability, while t sperm are fully functional (Bauer et al., 2005, 2007; Herrmann and Bauer, 2012; Lyon, 2003; Schimenti, 2000). In effect, t sperm are typically transmitted to about 90% of the offspring in heterozygous males.

1.2 Drive Suppressors: Mediating Genetic Conflict

We have seen that by violating Mendel's rules, driving elements are able to thrive in populations, often despite fatal fitness consequences for the organisms that harbour them. This is an unlikely end to the story: once the 'fair' rules of meiosis have failed at maintaining the integrity and function of the organism, we expect selection on the organism to evolve other measures to suppress the selfish action of the drive element. Argued from a gene-level perspective: both rival alleles at the same position in the genome, as well as the rest of the genome, suffer from the costs that a drive gene's selfish acts impose on the collective. As a reaction, we expect them to evolve countermeasures against the harmful effects of the driver.

We can conceptualize joint evolution of a drive gene and drive suppression by adding a second axis (measuring drive suppressor frequency) to our multilevel conflict picture illustrated in Figure 1.1. Accordingly, the co-evolutionary cycles of the conflict and conflict resolution can be viewed as a game 'played' in a two-dimensional trait space (gene drive and a suppressor) where selection acts at two levels (see schematic illustration in Figure 1.2). Selection for a drive suppressor may help the organism to avoid drive-related fitness costs, thus allowing the system to evolve back to a state that is beneficial to the organism as a whole (see Fig. 1.2). A multitude of such drive suppression mechanisms have been discussed in the literature. Just as gene drive itself, suppression of drive can act on different evolutionary levels.

Drive suppression at the genetic level The most immediate path to resolving the multilevel conflict from the viewpoint of the organism is the evolution of genes that suppress drive at the genetic level, through interrupting its drive mechanism or removing the target of its attack (see right column in Fig. 1.2 for a schematic illustration of a genetic drive suppressor). Theoretical models suggest that such drive suppressors should readily spread through drive-infected populations (Charlesworth and Hartl, 1978), and genetic suppressors of drive have been described in several systems, e.g. the *segregation distorter* (*SD*) in *Drosophila melanogaster* (Hiraizumi and Thomas, 1984) or the three *sex ratio* drivers (*SR*) in *D. simulans* (Atlan et al., 2003). Indeed, in recent years a sex chromosome driver in *D. simulans* has been observed to spread throughout the whole of Africa, only for a suppressor element to rapidly spread after it, suppressing the driver and normalizing sex ratios across the continent (Bastide et al., 2011). Once a drive suppressor reaches fixation, transmission ratios appear normal, and in the case of sex chromosome drive, sex ratios are returned to normal. Under

these circumstances, the presence of the functional drive allele can only be detected when animals are crossed with individuals from different geographical origins, where the suppressor gene is absent (Atlan et al., 2003).

Drive suppression at the organism level For reasons that are not understood, genetic suppressors of drive are —in spite of their effectiveness— not found in all drive systems. As a result, many other means to counteract drivers have evolved, e.g. mechanisms that reduce fitness of drive carriers at the organismal level. The conceptual logic of drive suppression at the organismal level is depicted in the right column of schematic Figure 1.2. Here, the suppressor does not modify genetic selection (i.e. gene drive itself), but *increases* the intensity of negative selection against organisms carrying the drive gene. Evolutionary forces that decrease the fitness of heterozygotes are particularly efficient, as they target the only place where drive actually can occur (van Boven and Weissing, 2001). Note that this is a common feature in all types of social conflict: selfish particles can typically only be effective if the group is *heterogeneous*, that is in the presence of other particles that can be exploited (in the logic of the example above: $Var(p_{ij}) > 0$). In the case considered here, the only heterogeneous groups available are heterozygotes. Thus, any mechanism that reduces the frequency of heterozygotes will deprive the driver of its main breeding ground. It may be worth noting that the logic underlying this argument is exactly analogous to Hamilton's idea of kin selection (Hamilton, 1964). Reducing the fraction of heterozygotes is equivalent to increasing the *statistical association* between same-type particles in a population. In the case of alleles considered here, this statistical association is typically measured by inbreeding coefficient F. In the classical case of kin selection, the statistical association among same-type particles is usually measured by the relatedness coefficient r. In both cases, an *increase* in the statistical association will result in a *reduction* in the frequency of the selfish particle (drive genes or selfish organisms), because cooperative particles typically do well when associating with same types, while selfish particles struggle if mainly clustered with other selfish partners.

Drive suppression at the population level Driving genes not only have major impacts on individual fitness, but they may also affect the success of populations or species. The drive allele may render large proportions of a population sterile or, in the case of sex chromosome drive, create populations with strongly biased sex-ratios (Bryant et al., 1982). In both cases, it is likely that the driver affects the per capita offspring production and hence the competitive ability of a population (Unckless and Clark, 2014), and in extreme cases could eliminate the entire population as a conse-

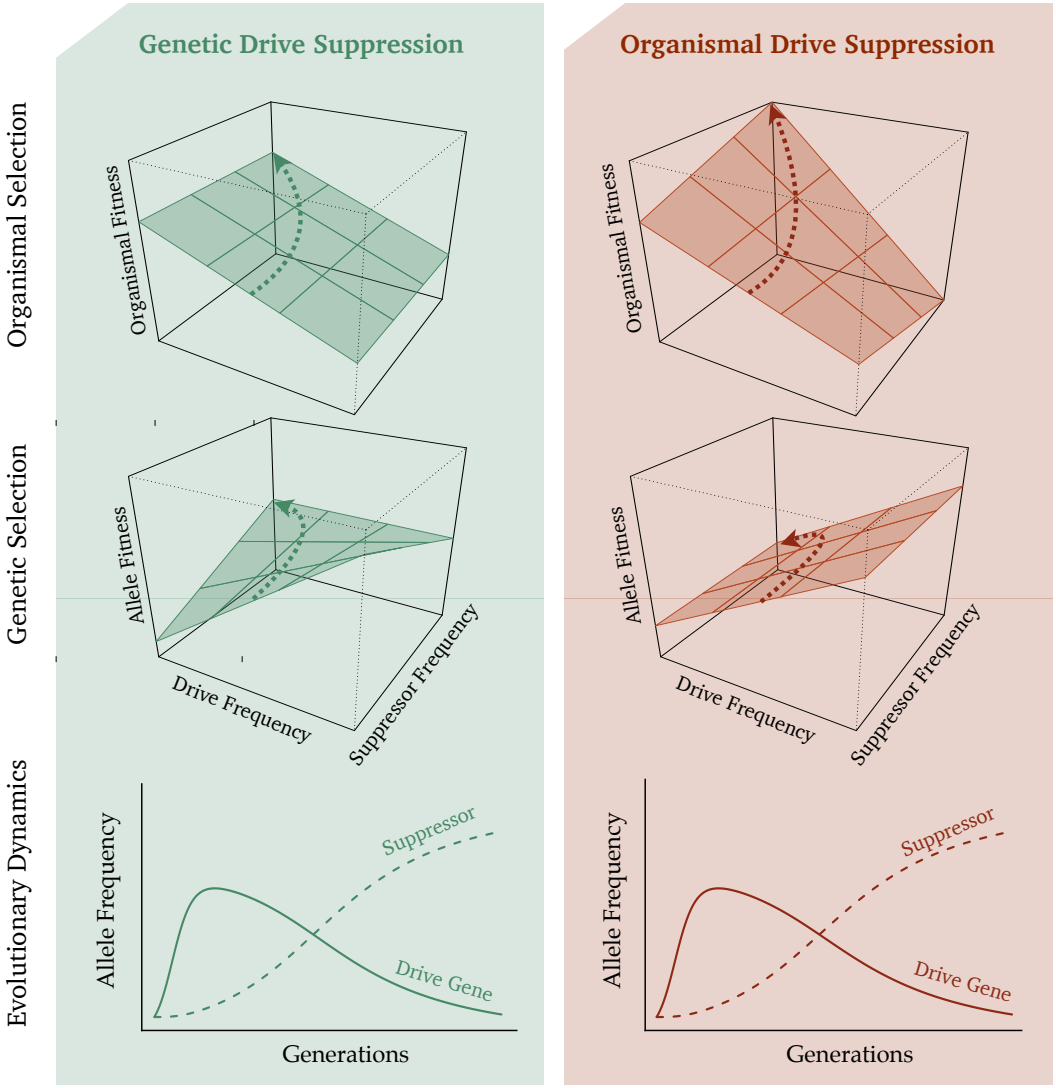

Figure 1.2. Schematic illustration of the evolution of gene drive and drive suppression considered throughout this thesis (simplified). Selection simultaneously acts along two axes (x-axis: drive gene; y-axis: drive suppressor) and at two hierarchical levels (top row: organismal selection; centre row: genetic selection). In the absence of a suppressor, the drive gene is evolutionarily stable at an intermediate frequency despite causing harm to the organism as a whole (as already shown in Fig. 1.1). As a result, we expect evolution of suppression mechanisms. Genetic suppressors of drive (left column) remove the drive allele's selective advantage within the organism (centre left panel). Drive suppression at the level of the organism will increase the selection intensity against drive carrying organisms (top right panel). In both cases, the system may evolve towards a state that is beneficial to the organism as a whole. The conflict is resolved (see top and bottom rows).

quence of a lack of one sex or complete sterility. Extinction through sex chromosome drive has been observed in laboratory populations (Price et al., 2010; Lyttle, 1977), and there is evidence that some well-studied populations may be nearing collapse in nature (Pinzone and Dyer, 2013). If drive can eliminate entire populations, it is possible that such higher order effects might play a role in the evolution and stability of drive (Nunney, 1993; Hatcher et al., 2000).

Drive Suppression Considered in this Thesis: Sexual Selection

A route to suppress the selfish acts of a drive gene that has received more attention in recent years is the evolution of female mating strategies that help them avoid fertilization by drive carrying males (Zeh and Zeh, 1996). We expect strong selection on females to avoid drive-carrying gametes, because it will protect her offspring from the often substantial, drive-related fitness costs. In the example of the *t* haplotype, $+/t$ females were shown to lose 40–50% percent (as expected with drive levels around 90%) of their offspring if mating with a +/t male only (Lindholm et al., 2013; Sutter and Lindholm, 2015). The incentive to evolve measures to avoid *t* fertilization is thus substantial. A systematic fertilization bias may arise from several processes, occurring at different stages of the mating process. Two strategies of female drive avoidance have received particular attention in the literature (reviewed in Wedell (2013)): Precopulatory mate choice and polyandry with subsequent sperm competition. Note that both mechanisms will, in effect, reduce the reproductive success of drive-carrying males. They may hence attributed to the class of organism-level drive suppressors.

Avoiding drive prior to mating The most obvious way for a female to avoid fertilization by a drive-carrying male is to avoid to mating with drive-carriers. There is only scarce evidence for such a precopulatory mating bias, the most prominent example being the *t* haplotype in house mice. In a series of experimental studies, drive-carrying female house mice were repeatedly shown to prefer drive-free males based on olfactory cues (reviewed in Lenington 1991). A second example stems from a sex-ratio distorter in stalk-eyed flies, where males that carry a genetic drive suppressor were shown to have longer eye stalks. Females typically prefer males with long stalks. In this case, the preference ensures that a female will produce males and females at equal proportions (Wilkinson et al., 1998).

Avoiding drive after mating Many drive systems occur in males and kill sperm that do not carry the driver. While this increases the transmission rate of the drive

chromosome to more than 50% of the offspring, it often results in drive males producing few viable sperm. Thus, drive may be successful within a male's ejaculate, but the few sperm of drive bearing males are often outcompeted by the more numerous sperm from non-driving males in sperm competition between males. Females may take advantage of that fact and systematically mate with several males (a phenomenon called polyandry), thereby avoiding fertilization by the drive male (Haig and Bergstrom, 1995). There is ample evidence for such connections between polyandry and gene drive from a broad range of taxa. Reports that gene drive favours polyandry have been provided —to only name a few— in *Drosophila simulans* (Atlan et al., 2004), *Drosophila pseudoobscura* (Price et al., 2008a), and the butterfly *Hypolimnas bolina* (Charlat et al., 2007).

The Ecological Consequences of Drive and Drive Suppression: Two Outstanding Questions

An in-depth understanding of the dynamic relationship between drive and drive suppression is worthwhile for (at least) two reasons. Schematic Figure 1.2 helps us to see why: we have seen that the cycles of drive and drive suppression can be conceptualized as a co-evolutionary arms race on a two-dimensional 'conflict plane', where selection occurs at several levels of the biological hierarchy (Fig. 1.2). In fact, we may not understand selection on one axis without considering the other. Connected to the two axes (drive and drive suppression) are two fundamental, interconnected questions. Both questions have provided invaluable insight and have been, and continue to be, at the very heart of ecological drive research (Burt and Trivers, 2006).

Research Question 1: What factors determine the frequency of drive genes in natural populations? In this first question, we focus on the drive-axis and ask how potential other factors may influence and help us explain the frequency dynamics of drive. Despite longstanding efforts, we still understand little about the factors that explain the spread and maintenance of drive genes in natural populations (Burt and Trivers, 2006). What are the conditions that allow drive genes to invade a population? Under what circumstances would we expect drive systems to be maintained in a population as a stable polymorphism? When would we expect drive systems to spread to fixation? How do suppressors affect the frequency of drivers in natural populations?

Research Question 2: How does gene drive affect the evolution of other traits? Understanding the course of genomic conflict and conflict resolutions may not only help us to understand the evolutionary dynamics of drive, but organismal function more generally. In this second question, we turn our focus on the two-dimensional conflict plane around and ask the reverse question: how do driving elements (and the genetic conflicts that go along with it) affect the evolution of other organismal features? As we have seen above, previous research suggests that integral features of eukaryote biology such as recombination and inactivation of DNA in gametes play an important role in drive suppression. Thus, understanding multilevel conflict and its resolutions can have far-reaching implications for the evolution of other important features of life. Importantly, these features may remain unexplained under the paradigm of individuals as the sole fitness maximizing agents. Consider the graphical representation of the conflict in schematic Fig. 1.1. The suppressor trait is only subjected to positive selection because the system is initially in a state that is sub-optimal for the organism. Without this perturbation, i.e. in a Mendelian scenario, there would not be the need for this trait evolution, because the population will already be at the population optimum.

1.3 Research Questions

Research questions 1 and 2 build the conceptual backbone of this thesis. The overall aim of this thesis is to understand the co-evolutionary relationship between gene drive (as the 'drive axis') and female mating behaviour (as the 'suppressor axis'). In particular, we are interested in how female mating behaviour affects drive frequency dynamics (RQ 1) and whether the presence of a drive gene can facilitate the evolution of particular female mating behaviours (RQ 2). Both questions are addressed using a variety of methodological approaches, ranging from theoretical modelling to laboratory experiments on wild house mice to data analyses on a natural population of wild house mice. Moreover, both questions can be related to a well-known evolutionary paradox.

Research Question 1 and the Low *t* Frequency Paradox

In this first question, we ask whether female mating behaviour can help us explain the frequency dynamics of the *t* haplotype in natural populations. In contrast to the

detailed knowledge of the structure and genetics of the t haplotype as outlined above, several key questions concerning its ecology and evolution remain unresolved (Burt and Trivers, 2006). For example, the factors that determine the frequency of t haplotypes in natural population is still very little understood, despite over half a century of theoretical and empirical research dedicated to this question (Ardlie and Silver, 1998). At the heart of this question lies a puzzle termed 'the t frequency paradox', which describes the discrepancy between theoretical t frequency predictions and empirical observations.

A theoretical prediction In 1957, Bruck (1957) provided a theoretical model that analysed t frequency dynamics in an infinitely large, unstructured population. The model incorporated the t haplotype's two main effects: drive and homozygote lethality (see above). Equation 1.1 helps us understand the model's main finding: the t haplotype will be positively selected at the within genotype level, while negatively selected at the between genotype level. Evolutionary change will stop at the point where selection at the two levels are in balance ($Cov(w_j, p_j) = -E[Cov(w_{ij}, p_{ij}]$). Hence, the model predicted a stable, internal equilibrium given by $\hat{p}_t = \frac{1}{2}\sqrt{\frac{\tau(1-\tau)}{\tau}}$. Drive levels in most t haplotype versions is around 90%. Bruck's parsimonious model predicts equilibrium frequencies in natural populations of $\hat{p}_t = 0.33$, a point where two thirds of the population are heterozygous $+/t$. Note that t frequency in this case cannot exceed 0.5, the point where all population members are $+/t$ heterozygous.

Empirical frequency observations The theoretical prediction is in marked contrast to t frequencies in natural and semi-natural mouse populations. Various studies measuring t haplotype frequencies in different mouse subspecies around the globe found t haplotypes at low, but stable levels, with frequencies ranging between 0.05 and 0.15 (Ardlie and Silver, 1998; Manser et al., 2011). Moreover, t frequencies are negatively correlated with population size, thus frequencies measured in small populations are typically larger.

Previous propositions to resolve the t frequency paradox The fact that frequencies are lower than expected based on the t's two main effects suggests the presence of additional forces that suppress it. A considerable number of predominantly theoretical studies are dedicated to the examination of evolutionary forces that might suppress the t, thereby accounting for the low, observed frequencies in a natural context. As already outlined above, the evolution of genes that directly interfere with drive at a molecular level would probably be the easiest and most direct path to t suppression. Surprisingly, there is little evidence for genetic drive suppressors in natural

populations of house mice. A whole series of computer simulation studies has analysed how genetic drift, population subdivision and inbreeding affect *t* haplotype frequencies (Levin et al., 1969; Lewontin and Dunn, 1960; Nunney, 1993; Petras and Topping, 1983). All these factors are known for their lowering effect on heterozygosity levels in a population. As a result, they typically did result in lower *t* frequency predictions. Furthermore, any form of selective $+/t$ heterozygote disadvantage, whether caused by differences in viability (Johnston and Brown, 1969; Lewontin, 1968; Young, 1967)), mating success (Lenington, 1991) or fertilization success (Manser et al., 2011), will act to reduce *t* frequencies in natural populations.

This thesis From the considerable body of theoretical work, we know that most of the above described evolutionary forces have the potential to resolve the *t* paradox. However, most propositions remain speculative because they rely on parameters for which solid estimates are largely missing (Ardlie and Silver, 1998; Burt and Trivers, 2006). In this thesis, we attempt to fill parts of this empirical gap by specifically quantifying key parameters under laboratory (chapters 3 and 4) and natural conditions (Chapter 6). As outlined above, we thereby specifically focus on the role of female mating behavior in the form of precopulatory mate choice or polyandry and sperm competition. The *qualitative* theoretical arguments as to how these two factors may suppress *t* frequency has already been developed previously. Here, we thus mainly make use of theoretical models to *quantitatively* test whether the parameter values as measured are in fact sufficient to account for the observed *t* dynamics (Chapters 4 and 5).

Research Question 2 and the Lek Paradox

In this second question, we turn the conflict plane by 90 degrees and ask whether the presence of a drive gene can facilitate the evolution of female mate choice and polyandry. Understanding the evolution of both female mate choice and polyandry is a long-standing and controversial focus of evolutionary research.

Males and females differ largely in their investment into gametes (termed anisogamy). This asymmetry in investment is widely regarded as the origin of sexual selection (Kokko et al., 2006): the smaller and more abundant sperm will typically compete for fertilization over the large and rare eggs. Given the male-male competition over fertilization, it is straight-forward to envisage rapid evolution for traits that give males an edge over their same-sex rivals (such as sperm that swims faster or weapons that

increase fighting ability; termed intra-sexual selection, Darwin (1871)). The question why females across the animal kingdom often undertake considerable time and energy costs to choose the right mating partner (inter-sexual selection), is far less obvious, and remains a controversial topic in evolutionary research (e.g. Andersson (1994)). Likewise, because sperm is available in abundance, we would expect that mating with one male would be sufficient to ensure female reproduction (Bateman, 1948). Consequently, it seems unclear why females would mate with several males (polyandry), especially considering that additional matings, too, are often energetically demanding and associated with large risk (such as the exposure to sexually transmitted diseases, e.g. Chapman et al. (1995)).

The 'good genes' and 'good sperm' hypotheses There is a wide range of hypotheses that explain why females exhibit mating preferences or promiscuity in spite of the supposed costs. For example, it has been hypothesised that females might choose males that carry traits which are indicative of his genetic quality. If this genetic quality is heritable, a choosy female could gain indirect fitness benefits by producing high quality offspring (termed 'good genes' or 'indicator models' of sexual selection, see for example Andersson 1994). An analogous argument has been put forward as an adaptive explanation for polyandry. If the sperm quality of a given male reflects a male's overall heritable genetic quality, the sperm of high quality males will typically win the sperm race. By 'inviting' sperm competition through polyandry, a female could ensure fertilisation by males that will transmit their high quality to her offspring (Yasui, 1997). As a result, polyandry may be beneficial despite potential costs.

The lek paradox There is a catch to 'good genes' and 'good sperm' arguments. Because they outcompete inferior males in sperm competition or during the mate choice process, males of high genetic quality will overall enjoy a larger mating and/or fertilisation success (Kokko, 2001). As a result of this positive, directional selection on high quality males, we would expect low quality males to be removed from the population. Thus, both processes are bound to end in a situation where there is no *variation* in male genetic quality. A population inhabited by high quality individuals only is probably beneficial for the population as whole. Yet there is no point in being choosy without a choice. In other words, if the population is depleted of variation in male quality, both choosy and randomly mating females gain the *same* indirect genetic benefits, because they are fertilized by the *same* males. In this situation, it is no longer adaptive to pay mate-choice costs, and as a result, we expect choice (or polyandry) to disappear from the population. This fascinating problem, generally known as the 'lek

paradox', is a long-standing problem in sexual selection research (e.g. Kirkpatrick and Ryan (1991)).

Resolutions to the paradox Several propositions have been provided as to how variation in male quality can be maintained in spite of directional sexual selection, thereby resolving the lek paradox. For example, mutations on male genetic quality may be biased and, as a result, predominantly act to decrease male quality (Pomiankowski et al., 1991; Pomiankowski and Moller, 1995). Moreover, classical sexual selection models typically assume that preference costs are fixed. Yet a (potentially) choosy female may not have to pay a preference-related fitness cost in a situation where she does not have to execute a choice (as is the case in the absence of variation in male quality). A recent study has theoretically explored this interesting idea and found that such variable mate-sampling costs can indeed explain the stably persistence of mate choice (Kokko et al., 2015).

This thesis In this thesis, I ask whether the presence of a driver can facilitate the evolution of costly female mate choice and polyandry. As outlined above, fertilization by drive males can have dramatic consequences on a female's offspring. Thus pre- and postcopulatory drive avoidance strategies are expected to be beneficial (e.g. Lande and Wilkinson (1999)). In Chapters 3–6, I provide empirical measures for the fitness consequences of female mating strategies under laboratory and natural conditions. Importantly, in the case of female drive-avoidance, the lek paradox may only play a minor role. We have seen above that gene drive can result in the *stable* maintenance of organisms that *differ* in their organismal-level genetic quality (Fig. 1.1). As a result, we may expect the disfavoured drive allele to be maintained despite directional sexual selection against it. In this thesis, I theoretically explore this idea in the context of precopulatory choice (Chapter 2). Potential selection on female mating behaviour will only generate an evolutionary response if the trait of interest is heritable. Consequently, I measured additive genetic variation for polyandry rates under natural conditions (Chapter 6).

Outline

Chapter 2 lays the conceptual foundation for the entire thesis. It investigates the coevolutionary dynamics between autosomal gene drive and female mate choice in purely theoretical terms. Although the model considers simultaneous evolution of both a drive gene and sexual selection, and thus touches on research question 1 and 2, it primarily

focuses on the latter. Hence, I investigate whether the presence of gene drive can facilitate the evolution of female mating preferences to avoid drive-carrying males, particularly in circumstances where such avoidance is associated with direct fitness costs to females.

In **Chapter 3**, I specifically test for the presence of female drive avoidance strategies under controlled laboratory settings, using the *t* haplotype system of house mice. Using a sophisticated mate choice apparatus, I gave female house mice the choice to freely visit and mate with either a $+/t$ or a $+/+$ male. This setup allowed me to not only test for the presence of social preferences for or against $+/t$ males during the testing period (as is usually the case in mate choice studies), but also to measure the paternity outcomes of a female's mating behaviour. In **Chapter 4**, I turn the focus more specifically to the postcopulatory mechanisms of drive avoidance. The study is based on a *post-hoc* analysis of an experimental evolution experiment on wild-caught Australian house mice performed by Renée Firman and Leigh Simmons, where selection lines where either kept under strictly monogamous or polyandrous mating conditions for 20 generations. Unknown at the time of the experiment, the *t* haplotype was present at considerable frequency in all eight selection lines, permitting me to test key predictions regarding polyandry as a drive suppression mechanism. Specifically, I measure how the *t* haplotype affected a male's sperm competitiveness, investigate how *t* frequencies are suppressed in the polyandrous selection lines, and compare the observed *t* frequency dynamics to theoretical model predictions. Hence, this chapter primarily deals with research question 1. In **Mini-Chapter 5**, we quantify the efficiency of polyandry as a drive suppressor for varying levels of sex-specific viability selection on $+/t$ heterozygotes. This allows us to explore the implications of gene drive and polyandry on intra-locus sexual conflict. Finally, **Chapter 6** takes a closer look at the role of polyandry in a natural population of house mice that has been monitored for over 15 years in unprecedented detail. I use this unique data set to investigate the genetic and environmental factors that influence the frequency of polyandry in the population. In particular, I address whether $+/t$ and $+/+$ females differ in their polyandry rates, and whether polyandrous behaviour is generally heritable. In a second step, I measure the impact of polyandry on female and male reproductive success, again with a particular focus on potential interactions with *t* genotype.

Chapter 2

The Evolution of Costly Mate Choice against Segregation Distorters

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In review (Evolution)

Abstract

The evolution of female preference for male genetic quality remains a controversial topic in sexual selection research. Conventional genetic mechanisms are usually insufficient to maintain variation in male quality. Such variation is, however, a key requirement for the evolution of costly female preference. Segregation distortion may be a mechanism to maintain variation in male genetic quality despite directional sexual selection. Here, we theoretically investigate a scenario where females pay a direct fitness cost to avoid males carrying an autosomal segregation distorter (i.e. mate choice for Mendelian inheritance). We show that the evolution of a costly preference is greatly facilitated under such circumstances, because (a) the distorter is maintained in population through segregation distortion and (b) females avoid fitness costs associated with the distorter. First, we consider the scenario where the male sexual signal and the distorter are genetically fully linked. In this case, female preference alleles readily spread to fixation. Interestingly, fixation does not occur if the female choice allele induces a very strong preference; such alleles drive the distorter to such low frequencies that the benefits of choosiness become negligible. Second, we study the situation where recombination can occur between the male signal and the distorter. We find that even small degrees of recombination do not allow the persistence of the costly preference. Hence, even in a system where the lek paradox does not play a major role, costly preferences can only spread under specific circumstances. In light of these results, we discuss the importance of distorter systems for the evolution of coslty female choice, both at a pre- and postcopulatory stage.

Keywords Sexual Selection, Mate Choice, Models/Simulations, Segregation Distortion, Meiotic Drive, Lek Paradox

2.1 Introduction

Directional sexual selection through female mate choice is likely to deplete genetic variation in male traits. If this occurs, genetic benefits of being choosy become small. This raises a simple yet puzzling question: why are females choosy if this choosiness depletes genetic variation in the male traits, which in turn is a prerequisite for the evolution of female choice? This fascinating question, generally known as the 'lek paradox', is a long-standing puzzle in sexual selection research (Kirkpatrick and Ryan, 1991).

Any resolution of this problem requires an explanation of how male trait variation persists despite directional sexual selection imposed by female choice. Several such explanations have been provided elsewhere (Pomiankowski et al., 1991; Pomiankowski and Moller, 1995; Kotiaho et al., 2001; Tomkins et al., 2004). Here, we want to theoretically examine the potential of segregation distorter systems to facilitate the evolution of costly female mate choice. By distorting transmission ratio in their own favor, distorters may act as generators of allelic variation in the male trait. In consequence, genetic variance in the trait may be maintained despite directional sexual selection. Moreover, distorters are usually associated with substantial fitness costs to their carriers (Burt and Trivers, 2006). Females may hence protect their offspring from detrimental fitness effects by avoiding fertilization with distorter-carrying males.

Connections between female choice and segregation distorters have been suggested by many empirical studies (see Wedell (2013) for a recent review). Female choice may happen both at a pre- and postcopulatory stage. Precopulatory preferences for an absence of distorters or for drive suppressors have been reported in stalk-eyed flies (Wilkinson et al., 1998; Cotton et al., 2014), house mice (Lenington et al., 1992), and *Drosophila paulistorum* (Miller et al., 2010). A larger body of work highlights the importance of mating biases at the postcopulatory stage. As a direct consequence of segregation distortion, distorter carrying males are typically weak sperm competitors (Zeh and Zeh, 1997). Hence, female multiple mating (polyandry) has been proposed as a possible female counterstrategy against distorters (Haig and Bergstrom, 1995). Polyandry will lead to systematic deviations from random mating assumptions. It has thus been considered a form of indirect female mate choice (Brooks and Griffith, 2010). Evidence for distorters favouring polyandry, to only name a few, have been found in *Drosophila simulans* (Atlan et al., 2004), *Drosophila pseudoobscura* (Price et al., 2008b), and the butterfly *Hypolimnas bolina* (Charlat et al., 2007).

Given this considerable body of empirical evidence, surprisingly few studies have investigated the theoretical implications of segregation distortion on mating preferences. However, sexual selection models are complicated considerably when a distorter is added. While most population genetics models of sexual selection are framed in terms of haploids (Kuijper et al., 2012), segregation distortion requires the analysis of diploid organisms, which makes analysis much more intricate (Greenspoon and Otto, 2009). Most previously published models focus on the interplay between female choice and sex-linked distorters. Motivated by the stalk-eyed fly system (Wilkinson et al., 1998), two models investigated possible interactions between female choice and a sex-linked distorter. Reinhold et al. (1999) consider female choice for a distortion suppressor. The model suggests that, unexpectedly, female preferences in favour of a distortion suppressor is always selected against. Lande and Wilkinson (1999) chose a more direct approach and analyzed a situation where females choose a male trait (eyespan in this particular example) that indicates the absence of the distorter allele. They found that female preference for the trait can evolve, but only if the trait is perfectly coupled with the distorter. Even a small rate of recombination between a trait locus and the distorter locus will prevent the evolution of female choice. Randerson et al. (2000) investigated the evolution of costly male mate choice in the butterfly *Acraea encedon* infected with male-killing *Wolbachia*. Because the male killer causes a strong female bias in infected populations, sex-roles appear reversed and males should avoid infected females. The model confirms this expectation, as long as males do not perfectly discriminate between infected and uninfected females. In this case, costly male choice can stably persist. If males make no mistakes, costly male choice succumbs to its own success, since by effectively removing the male killer from the population, it also removes the benefits of being choosy.

Here, we investigate a model for the evolution of a costly female preference in the presence of an autosomal segregation distorter. In particular, we address the following questions: (1) Can the presence of an autosomal distorter facilitate the spread of a costly female preference for Mendelian segregation (i.e. distorter-free males)? (2) What levels of preference cost, preference and distortion strength allow for the evolution of costly female preferences? (3) Are there systematic differences between sterile and lethal distorter types? (4) How does recombination between a male sexual signal and a distorter affect evolutionary outcomes?

2.2 The Model

Our model follows the standard set-up of population genetic models of sexual selection (Kuijper et al., 2012) and adds segregation distortion as an additional factor. We consider diploid organisms and three autosomal loci: a trait locus T encoding for a sexual ornament in males; a preference locus P affecting female choice for the ornament; and a distorter locus S affecting Mendelian segregation in males. The following two alleles segregate at each of the three loci (see Table 2.1 for an overview).

- The trait locus (T) is expressed in males only and encodes a trait that is subject to both viability and sexual selection. It contains alleles T_0 and T_1 (at frequencies t_0 and t_1, respectively), where allele T_1 induces a viability disadvantage but can be the target of female preference.

- The preference locus (P) is expressed in females only and determines her relative tendency to mate with males of the three possible genotypes at the T locus. It contains alleles P_0 and P_1 (at allele frequencies p_0 and p_1, respectively). The expression of female preference is associated with a fixed viability cost.

- The distorter locus (S) contains alleles S_0 and S_1 (at allele frequencies s_0 and s_1, respectively). The proportion of distorter alleles S_1 transmitted to the next generation in S_0S_1 heterozygote males is given by parameter d, ranging from $d = 0.5$ (Mendelian segregation) to $d = 1$ (complete distortion). Fitness effects of the distorter are inspired by the t haplotype system in house mice, where —depending on the distorter type— S_1S_1 homozygotes suffer either from male sterility (sterile type) or lethality in both sexes (lethal type).

The life cycle We consider an infinite population of diploids with non-overlapping generations. Because males and females are differently affected by selection, we track their genotype frequencies independently. Let $X_{ij,kl,mn}$ denote female genotype frequencies, where ij defines status at the T locus, kl status at the P locus, and mn status at the distorter locus S. Analogously, male genotype frequencies are given by $Y_{op,qr,st}$. To derive the recursion equations for the resulting 64 ordered male and female genotypes, we assume the following life cycle.

We start our life cycle with the zygotes of the present generation. Analogous to above, the sex-independent genotype frequencies at the zygote stage are given by

Trait locus T		T_0T_0	T_0T_1	T_1T_1
Viability (♂)		1	$1-h_tc_t$	$1-c_t$
Preference locus P		P_0P_0	P_0P_1	P_1P_1
Preferences (♀)	T_0T_0	1	1	1
	T_0T_1	1	$1+h_ah_pa$	$1+h_aa$
	T_1T_1	1	$1+h_pa$	$1+a$
Viability (♀)		1	$1-c_p/2$	$1-c_p$
Segregation locus S		S_0S_0	S_0S_1	S_1S_1
either: Viability (♂♀)		1	1	0
or: Fertility (♂)		1	1	0
Segregation ratio (♂)		0	d	1

Table 2.1. Overview over the three loci and the parameters used in the model. Sex symbol in brackets indicate the sex in which the given property is expressed.

$Z_{ij,kl,mn}$. First, viability selection occurs. Viabilities are different in the two sexes (see Table 2.1). Females carrying P_1 alleles suffer from a fixed viability cost c_p (cost of preference). For simplicity, we assume that viability selection at the preference locus is additive (viability of P_0P_1 heterozygotes is $1-\frac{c_p}{2}$). Likewise, the male trait may come at a viability cost c_t. Here, T_0T_1 heterozygote fitness is given by dominance coefficient h_t (viability of T_0T_1 heterozygotes is $1-h_tc_t$). In the case of a distorter with homozygous lethal effects, S_0S_0 individuals have zero viability irrespective of sex. The resulting overall viabilites for males $w_{ij,kl,mn}$ and females $v_{ij,kl,mn}$ are then given as the product of the viability effects at each locus. Based on the zygote frequencies $Z_{ij,kl,mn}$, we can calculate the adult genotype frequencies:

$$X_{ij,kl,mn} = Z_{ij,kl,mn}\frac{v_{ij,kl,mn}}{\bar{v}}, Y_{op,qr,st} = Z_{ij,kl,mn}\frac{w_{ij,kl,mn}}{\bar{w}}, \tag{2.1}$$

where $\bar{v}$ and $\bar{w}$ denote mean female and male viability, respectively.

In the second step, adults of the present generation mate with each other. Females choose mates according to fixed relative preferences. This relative tendency of a female of P-genotype kl to mate with a male of T-genotype op is given by $a_{kl\times op}$ (see also Table 2.1). Parameters h_p and h_a describe dominance effects of preference, where h_p defines preference strength *of* P_0P_1 heterozygote females and h_a quantifies preference strength *for* heterozygote T_0T_1 males. The mating frequency between males of genotype op,qr,st and females of genotype ij,kl,mn is thus

$$F_{ij,kl,mn\times op,qr,st} = X_{ij,kl,mn} Y_{op,qr,st} \frac{a_{kl\times op}}{\overline{a_{op}}}, \tag{2.2}$$

where $\overline{a_{op}}$ is a normalizing constant that ensures that the fertility of a female does not depend on her mate choice.

Given the frequencies of the mating combinations from equation (2), we can now calculate the the resulting zygote frequencies $Z'_{ij,kl,mn}$ of the next, non-overlapping generation. Zygote frequencies will depend on segregation distortion d as well as on the recombination rate r_{UV} between loci U and V (r_{TS}, r_{PS}, r_{TP}). These recombination rates are not independent of each other, i.e. for a given combination of r_{TS} and r_{PS}, $r_{TP} = r_{TS}r_{PS} - 2r_{TS}r_{PS}$. In the case of a sterile distorter, matings involving S_0S_0 males produce no offspring.

All results presented in this manuscript reflect numerical solutions of the system of recurrence equations. Distorter frequencies are usually empirically measured at the adult stage. Allele frequencies in this manuscript were hence recorded at the adult stage. At this stage, we also calculated the standardized linkage disequilibrium D_{uv} between allele U_1 and V_1 (at frequencies u_1 and v_1) defined as (Lewontin, 1964)

$$D'_{uv} = \frac{D_{uv}}{D_{max}} \quad \text{where} \quad D_{uv} = uv_1 - u_1v_1 \quad \text{and} \quad D_{max} = \begin{cases} \min[u_0v_1, u_1v_0] & \text{if } D_{uv} \geq 0 \\ \min[u_0v_0, u_1v_1] & \text{if } D_{uv} < 0. \end{cases} \tag{2.3}$$

Here, uv_1 denotes the frequency of U_1V_1 haplotypes among adult genotypes.

2.3 Results

Evolution in the Absence of a Distorter

We begin our model analysis by considering sexual selection for a costly male trait in the absence of a distorter locus. The evolutionary outcome strongly depends on whether female preferences are cost-free (Fig. 2.1a) or whether choosiness is associated with costs (Fig. 2.1b).

Evolution of cost-free preference In the absence of a distorter, the evolution of cost-free female preferences ($c_p = 0$) has been studied in detail both numerically (Heisler and Curtsinger, 1990) and analytically (Gomulkiewicz and Hastings, 1990; Otto, 1991; Greenspoon and Otto, 2009). The evolutionary dynamics strongly resemble its haploid equivalent, Kirkpatrick's classical model of Fisherian sexual selection (Kuijper et al., 2012). Because there is no direct selection on the preference allele, p_1 evolves as a correlated response to changes at the trait locus (Fisher process). Evolution at the trait locus is determined by the interplay between natural selection (favouring allele T_0) and sexual selection (favouring allele T_1). Natural and sexual selection balance each other at points that form curves of quasi-equilibria in allele frequency space (the red curves in Fig. 2.1a); these curves correspond to the lines of equilibria in Kirkpatrick's haploid model. In the diploid model, some (very slow) movement along the curves of quasi-equilibria is possible, due to a change in genetic associations (Greenspoon and Otto, 2009). While in Kirkpatrick's model the line of equilibria is always attracting, this is not necessarily the case in the diploid model. As shown by Greenspoon and Otto (2009), the curve of quasi-equilibria can either be repelling (upper panel in Fig. 2.1a) or attracting (lower panel in Fig. 2.1b), depending on whether the combination of natural and sexual selection induces net underdominance or net overdominance at the male trait locus.

Evolution of costly preference—the lek paradox As in Kirkpatrick's haploid model, any female preference allele will eventually be selected against and disappear from the population if the slightest costs of choosiness are associated with this allele (Pomiankowski, 1987). In particular, two factors limit the evolution of a costly preference. (i) In case of a repelling curve of quasi-equilibria (2.1a), one of the alleles at the male trait locus will be driven to fixation and, as a result, male trait variation $var(t) = t_1 t_0$ will be exhausted. If the choosiness is costly (Fig. 2.1b, upper panel), the preference allele will be selected against when the trait locus is close to fixation (since

there are no benefits of being choosy in a nearly monomorphic population), and it will eventually be driven to extinction. In the literature, this problem is referred to as the 'lek paradox'. (ii) In case of an attracting curve of quasi-equilibria, evolution will drive the allele frequencies first close to this curve (Fig. 2.1b, lower panel). Once the curve is reached, however, there are again no indirect benefits of being choosy, since none of the male trait alleles is selectively favoured. As a consequence, even small choice costs induces selection against the preference allele, which, again, will disappear from the population.

The Distorter as a Target of Female Preferences

In the scenario considered above, a costly preference could not evolve because the system evolves to a state, i.e. no male trait variation under scenario (i) or equal male fitness under scenario (ii), where the benefits of choosiness become negligible. As a consequence, even small costs of choosiness become a dominant factor, leading to the disappearance of the preference allele. If female preferences are targeted at a distorter allele, the situation may be different. Distortion may (i) help maintain trait variation $var(t)$ despite directional sexual selection and (ii) confer benefits to choosy females even if trait alleles are at a polymorphic equilibrium.

It is unlikely that females base their mate choice directly at the males' genotype at the distorter locus. Instead, female preferences will be based on male traits that may convey information on the presence or absence of distorter alleles. Yet, we will postpone the analysis of such a three-locus scenario (distorter locus, trait locus, preference locus) and first consider the much simpler case where females can directly differentiate between distorter genotypes, or, equivalently, where the trait allele T_1 is in full linkage to the wildtype allele S_0 ($LD'_{ts} = -1$) and no recombination between the T and the S locus occurs ($r_{TS} = 0$). Thus, the model reduces to a diallelic 2-locus system, containing P_0 and P_1 alleles at the P locus and T_1S_0 and T_0S_1 haplotypes at the trait/distorter locus (henceforth, we will refer to distorter frequency s_1 only, where $s_1 = t_0 = 1 - t_1$). Because T_1 alleles only occur together with the wildtype S_0 allele, a female that chooses a T_1 male will, at the same time, avoid the distorter allele S_1.

We will first consider an illustrative example of mate choice targeted at a sterile distorter allele, based on the parameter values of Fig. 2.1b. Next, we investigate systematically how evolutionary dynamics are affected by model parameters and the type of distorter. Finally, we explain the various outcomes by means of a simple intuitive

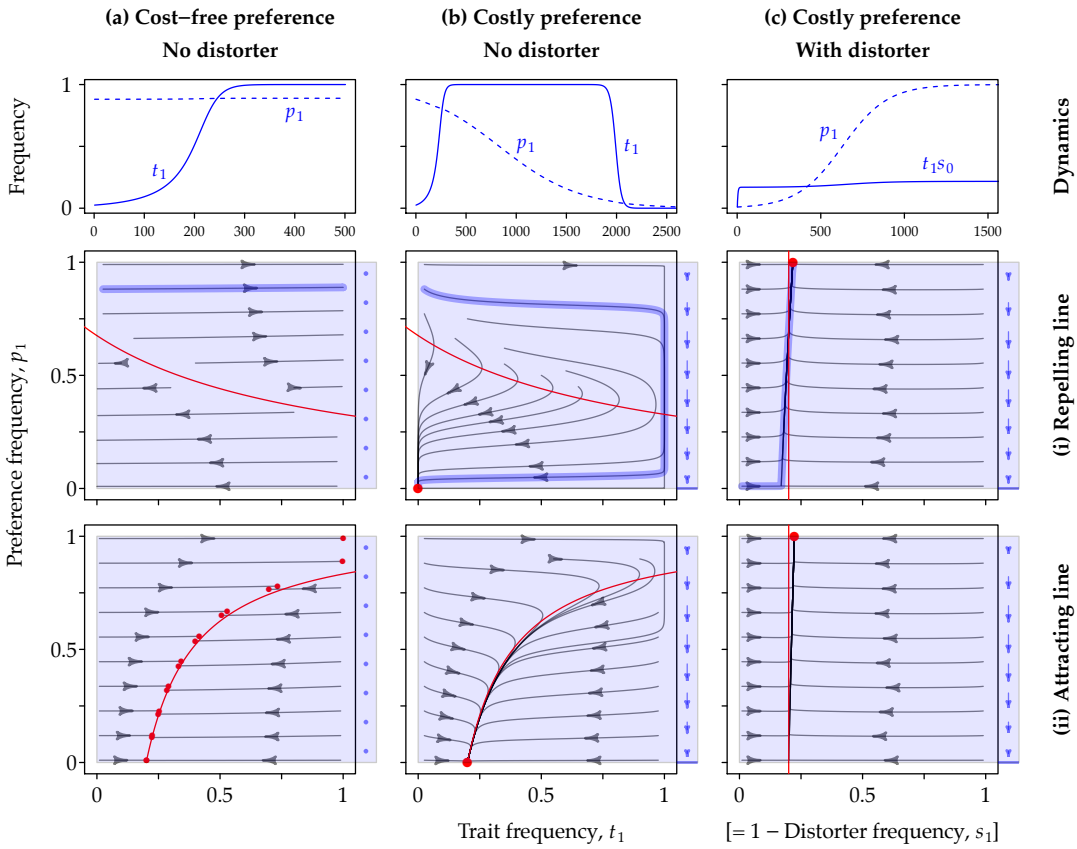

Figure 2.1. Joint evolution of trait t_1 and preference p_1 alleles in the absence (a–b) and presence (b) of a distorter. Center row panels illustrate (i) a scenario of a repelling line of quasi-equilibria, lower row panels (ii) a scenario an attracting line of quasi-equilibria (indicated by the red lines, based on Greenspoon and Otto (2009)). Top panels follow the allele frequency dynamics of a specific evolutionary trajectory of scenario (i) over time (shaded in blue). In (a), the preference is cost-free (parameter values for scenario (i): $a = 0.4$, $h_a = 0.5$, $h_p = 0.3$, $p_c = 0$, $c_t = 0.15$, $h_t = 0.5$; parameter values for scenario (ii): $a = 0.4$, $h_a = 0.5$, $h_p = 0$, $p_c = 0$, $c_t = 0.2$, $h_t = -1/3$). In (b), a preference cost $c_p = 0.005$ is added, resulting in the collapse of the quasi-neutral curves to a single, attracting point, where the preference allele is absent. In (c), the preference is targeted at a sterile distorter ($d = 0.9$, the remaining parameter values are identical to (b)). Now, the preference allele rises to fixation. The red vertical line indicates the distorter equilibrium in the absence of preference ($\hat{s}_{p=0}$). The blue arrows on the right show selection on preference alleles in the absence of a distorter/male trait ($p_1 = 0$). The red dots indicate the end points (equilibria) of each evolutionary trajectory.

argument. This will help us understand four qualitatively different evolutionary outcomes and their parameter dependence.

An illustrative example We start with a situation where females avoid a distorter that is selectively neutral in females and induces sterility in males that are homozygous for the distorter (as in case of the 'sterile t haplotypes' in the house mouse, Lyon (1986)). The evolutionary dynamics of sterile, autosomal distorters in the absence of sexual selection have been derived by Dunn and Levene (1961): the distorter is positively selected at the genetic level (segregation distortion) while counterselected at the organismic level (male sterility). The two forces balance at a stable, polymorphic equilibrium given by $\hat{s}_{p=0} = 2d - 1$ (see red vertical line Fig. 2.1c).

Figure 2.1c shows the evolutionary dynamics if the costly preference is targeted at a distorter. The parameter values are identical to the two scenarios in Fig. 2.1b, allowing us to directly compare the evolutionary outcome in the presence and absence of a distorter. The costly preference allele P_1 now rises to fixation, both in the repelling and attracting scenario. The two factors that previously inhibited the spread of costly preference are now avoided. Firstly, in contrast to the repelling scenario (i) in Fig. 2.1b, the distorter allele S_1 is not lost despite directional sexual selection against it. Sexual selection against the distorter is counteracted by segregation distortion favouring the distorter. Note that selection for distorter alleles S_1 is particularly strong at low distorter frequencies (van Boven and Weissing, 2001; Weissing and van Boven, 2001). The resulting polymorphism prevents the lek paradox and fuels selection at the preference locus. Secondly, in contrast to the attracting scenario (ii) in Fig. 2.1b, choice is beneficial even if the distorter frequencies are at the polymorphic equilibrium $\hat{s}$. Segregation distortion creates a situation where both S_1 and S_0 stably coexist, even though S_0S_0, S_0S_1 and S_1S_1 males dramatically differ in their individual fitness. The costly preference helps females to avoid the fitness costs of mating with distorter-carrying male.

Dependence of preference frequency on model parameters To systematically explore the parameter conditions that facilitate the evolution of a costly preference targeted at a distorter, we calculated evolutionary trajectories for systematically varying levels of preference strength a, preference cost c_p, and distortion strength d. Each model run was started with a low preference frequency $p_1 = 0.01$ and the distorter at equilibrium ($s_1 = \hat{s}_{p=0}$). With these starting conditions, we iterated the recurrence equations until allele frequencies reached equilibrium ($\hat{p}_1, \hat{s}_1$), defined as the point where allele frequency changes became exceedingly small (Δp_1 and $\Delta s_1 < 10^{-8}$).

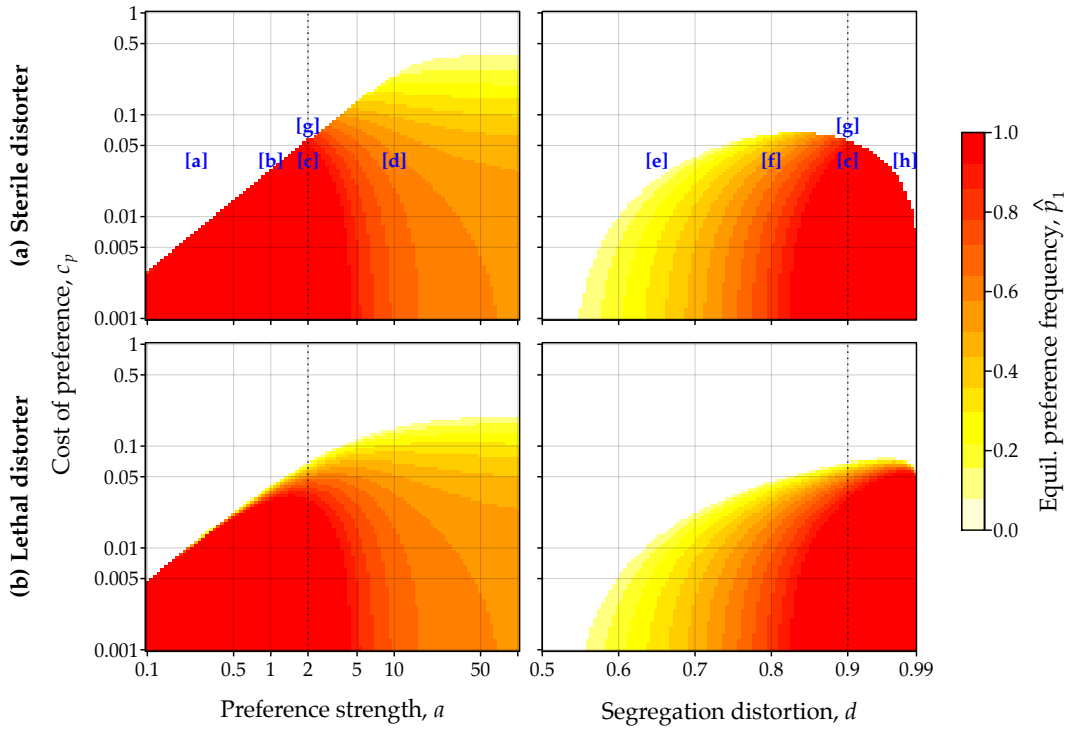

Figure 2.2. Equilibrium preference frequencies $\hat{p_1}$ of a preference allele targeted at (a) a sterile distorter and (b) a lethal distorter in relation to preference strength (a), preference cost (c_p) and distorter strength (d). Preference strength a and cost c_p are shown on a log_{10}-scale. Left panels are based on a distorter strength of $d = 0.9$, right panels on a preference strength of $a = 2$, with the vertical dotted lines indicating the location where phase-plots intersect. Letters a–e correspond to the position of the evolutionary trajectories shown in Fig. 2.4. Remaining parameter values: $c_t = 0$, $h_p = 0.5$, $h_a = 0$.

For simplicity, we assume that trait costs are absent ($c_t = 0$) and females do not differentiate between S_0S_1 and S_1S_1 males, i.e. they avoid them with same probability ($h_a = 0$).

Fig. 2.2 shows equilibrium preference frequencies $\hat{p}_1$ as a function of a, c_p, d, and the distorter type (sterile vs. lethal). Overall, the preference allele can invade and persist in a population for a large spectrum of the parameter space considered, both if targeted at a sterile or lethal distorter. In extreme cases, the preference allele can sustain preference costs as high as $c_p \approx 0.4$, i.e. a 40% viability reduction in choosy females. As one would expect intuitively, higher preference costs c_p invariably result in reduced preference frequency. Surprisingly, both preference strength a and distortion

strength d affect equilibrium preference frequencies in a non-linear fashion. Preference frequencies are highest at intermediate values of a and d. At low and high levels of d and a, the spread of a costly choice is typically limited (see following sections for an intuitive explanation of these results).

Dependence of preference evolution on distorter type The two different distorter types (sterile vs. lethal) generate only minor, qualitative differences in the evolutionary outcome. As is the case with sterile distorters, recessive lethal distorters induce a stable, polymorphic equilibrium given by $\hat{s}_{p=0} = \frac{1}{2} - \frac{\sqrt{2d-1}}{2d}$ (Bruck, 1957). If at all, the range of parameter values allowing the spread of the costly preference allele P_1 is slightly smaller in case of a lethal distorter when compared with sterile distorters. This can be understood if one considers that the female benefits of avoiding distorter carriers are slightly different for lethal and sterile distorters. In the case of a lethal distorter, the benefits are straightforward: a female avoiding distorter-carrying males prevents lethality in her progeny. In the case of sterile distorters —at least as it is implemented in the model here— benefits are twofold. First and foremost, a choosy female avoids S_1S_1 homozygotes and hence complete failure of reproduction due to male sterility. Second, she avoids S_0S_1 heterozygote males, which would render a potentially large proportion (depending on her own S genotype) of her male offspring sterile. It is this twofold advantage that may explain why costly preferences targeted at a sterile distorter evolve under a slightly broader parameter range.

A systematic analysis of parameter dependence To intuitively understand how the model parameters considered in Fig. 2.2 affect evolutionary outcomes, let us schematically examine two ranges of distorter frequency. Firstly, we specify the range of distorter frequencies that can be attained (at equilibrium) for varying frequencies of the preference allele, denoted as the 'feasible distorter frequency range' $[\hat{s}_{p=0}, \hat{s}_{p=1}]$. Secondly, we specify the distorter frequency range for which the preference allele is selectively favoured, denoted as the 'preference favouring range' $[s^-, s^+]$. Knowing how model parameters affect (1) the distorter range that can be attained and (2) whether these frequencies selectively favour the preference allele will help us to understand different evolutionary outcomes.

Fig. 2.3 illustrates the two ranges and their parameter dependence. The feasible distorter range (red area in Fig. 2.3) will fall between the distorter equilibrium where preference is absent ($\hat{s}_{p=0}$) and the distorter equilibrium where all females in a population are choosy ($\hat{s}_{p=1}$). The position of $\hat{s}_{p=0}$ is a function of the distortion strength d (see above), whereas the position of $\hat{s}_{p=1}$ is both a function of preference strength

(with large values of a increasing the range size) and distorter strength (with large values of d decreasing the size of the range, as sexual selection affects the distorter equilibrium only weakly if distortion is strong). The preference favourable range, on the other hand, spans the distorter frequency range where the costly preference allele is selectively favoured, i.e. were choice benefits outweigh the costs. Preference costs, as implemented in the model, are distorter frequency independent (see blue lines in Fig. 2.3). Preference benefits, on the other hand, crucially depend on distorter frequency: if the distorter allele is absent ($s_1 = 0$) or fixed ($s_1 = 1$) a female will gain no benefits from choice. Intermediate distorter frequency confer highest benefits (see black lines in Fig. 2.3). As a result, the preference allele will only be selectively favoured in the range $[s^-, s^+]$ (grey area in Fig. 2.3). The points s^- and s^+ mark the unstable and stable preference equilibria, respectively, where preference costs and benefits are in balance. Higher preference costs c_p reduce the size of the range, up to a point where costs outweigh benefits for all distorter frequencies. A larger preference strength a makes choice more effective and thus increases range size.

Depending on model parameters, the feasible distorter range and preference favouring range can be arranged in seven different ways (scenario 1–7, summarized in Table 2.2), corresponding to four qualitatively distinct evolutionary outcomes (see Fig. 2.4). In general, the evolution of the costly preference allele is limited, whenever the feasible distorter frequencies fall outside the preference favouring range.

Weak and strong preferences limit the evolution of costly choice In light of schematic Fig. 2.3, let us examine the non-linear dependence of preference equilibria $\hat{p}_1$ on preference strength a (as observed in Fig. 2.2). We have seen that larger values of a will increase the size of both the feasible distorter and preference favouring distorter range. Fig. 2.4a–d shows evolutionary outcomes for varying preference strengths a, keeping distortion strength d and preference costs c_p constant. If preference strength is very small/ineffective (a_{low}), preference costs either outweigh benefits for all distorter frequencies (scenario 1, see Fig. 2.4a) or the preference favouring range falls completely outside the feasible distorter range (scenario 2). As a consequence, the preference allele is lost irrespective of starting frequencies. If preference strength is increased slightly, the feasible distorter frequencies and preference favouring range partly overlap (scenario 3, see Fig. 2.4b). Thus, the preference allele is selectively favoured in one part of the feasible distorter range, but selectively disfavoured in the other. Depending on the starting conditions, distorter frequencies either end up above or below the unstable equilibrium point s^-. Accordingly, the preference allele

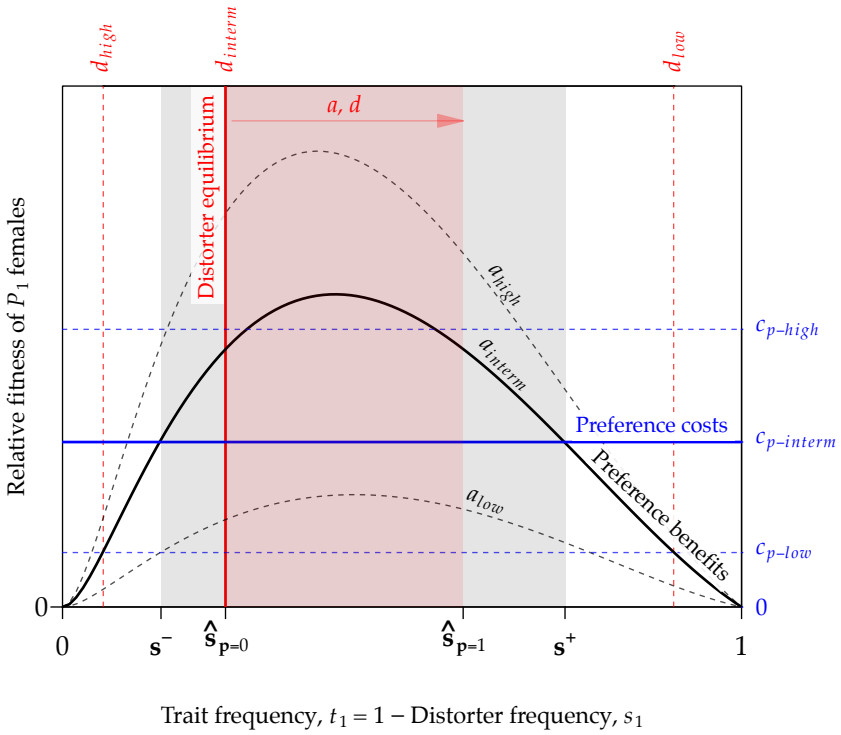

Figure 2.3. Schematic illustration of the feasible distorter frequency range (area shaded in red) and the preference favouring range (area shaded in grey) and their parameter dependence. The feasible distorter range corresponds to the spectrum of the distorter equilibria, ranging from the distorter equilibrium where preference is absent $\hat{s}_{p=0}$ to the distorter equilibrium where all females express a preference $\hat{s}_{p=0}$. The red vertical line show the position of $\hat{s}_{p=0}$ for low, intermediate, and high levels of distorter strength d. The position of $\hat{s}_{p=1}$ will be a function of both preference and distorter strength a, d. The preference favouring distorter range specifies the distorter frequency spectrum where preference allele is selectively favoured, i.e. where preference benefits outweigh preference costs. The blue horizontal lines illustrate the effect of low, intermediate and high levels of preference costs c_p on the relative fitness of the preference allele. Note that preference costs are distorter frequency independent. The black curves indicate preference benefits for low, intermediate and high levels of preference strength a. The points where the cost and benefit line intersect, i.e. where preference costs and benefits are in balance, correspond to unstable and stable preference equilibria s^- and s^+, respectively.

either goes to fixation or is lost. At intermediate preference strength (a_{interm}), the feasible distorter range is entirely included in the preference favouring range (scenario 5, see in Figs. 2.3 and 2.4c). As a result, the preference allele is selectively favoured for all distorter equilibria and goes to fixation irrespective of starting frequency. Interestingly, if preference strength is further increased (a_{high}), sexual selection will at some point become strong enough to push the distorter equilibrium $\hat{s}_{p=1}$ outside the preference favourable range, such that $\hat{s}_{p=1} > s^+$ (scenario 6). This scenario often results in damped oscillatory dynamics, as shown in Fig. 2.4d. First, sexual selection drives the distorter allele S_1 close to extinction. As a result, the costs of choosiness start to outweigh its benefits, which will result in a decrease in preference allele frequency p_1. A decreasing proportion of choosy females will in turn reduce sexual selection against distorters and s_1 moves back into the preference favouring range. This causes, once more, an increase in preference frequency and the cycle starts anew. The process finally comes to a halt at the stable, internal equilibrium s^+, where costs and benefits of the preference allele are in balance.

Weak preferences and strong distorters limit the evolution of costly choice

Distorter strength d also affects equilibrium preference frequencies in a non-linear fashion (as shown in Fig. 2.2). Schematic Fig. 2.3 can again help us to intuitively understand this relationship. Parameter d alters both position and size of the feasible distorter range. Figs. 2.4e,f,c,g depict evolutionary dynamics for different distorter strength d, keeping preference strength a and costs c_p constant. If distorter strength is very high (d_{high}), the distorter equilibrium $\hat{s}_{p=0}$ will be close to one and the preference favouring range will be small. As a consequence, the feasible distorter range will likely be located outside the preference favouring range (scenario 2, see Fig. 2.4g). Intermediate to high distorter strengths (d_{interm}) result in intermediate distorter frequencies, the conditions most favourable for the costly preference allele (scenario 5, Fig. 2.4c). If distorter strength is weak (d_{low}), the distorter equilibrium $\hat{s}_{p=0}$ will be low and the feasible distorter range large, resulting in scenario 6 (Fig. 2.4f) and 7 (Fig. 2.4e). In Fig. 2.4g, preference costs c_p are slightly elevated. It illustrates scenario 4, where the feasible distorter range is included in the preference favourable range. Hence, depending on starting conditions, the preference frequency goes to zero or ends up at the stable, internal equilibrium s^+.

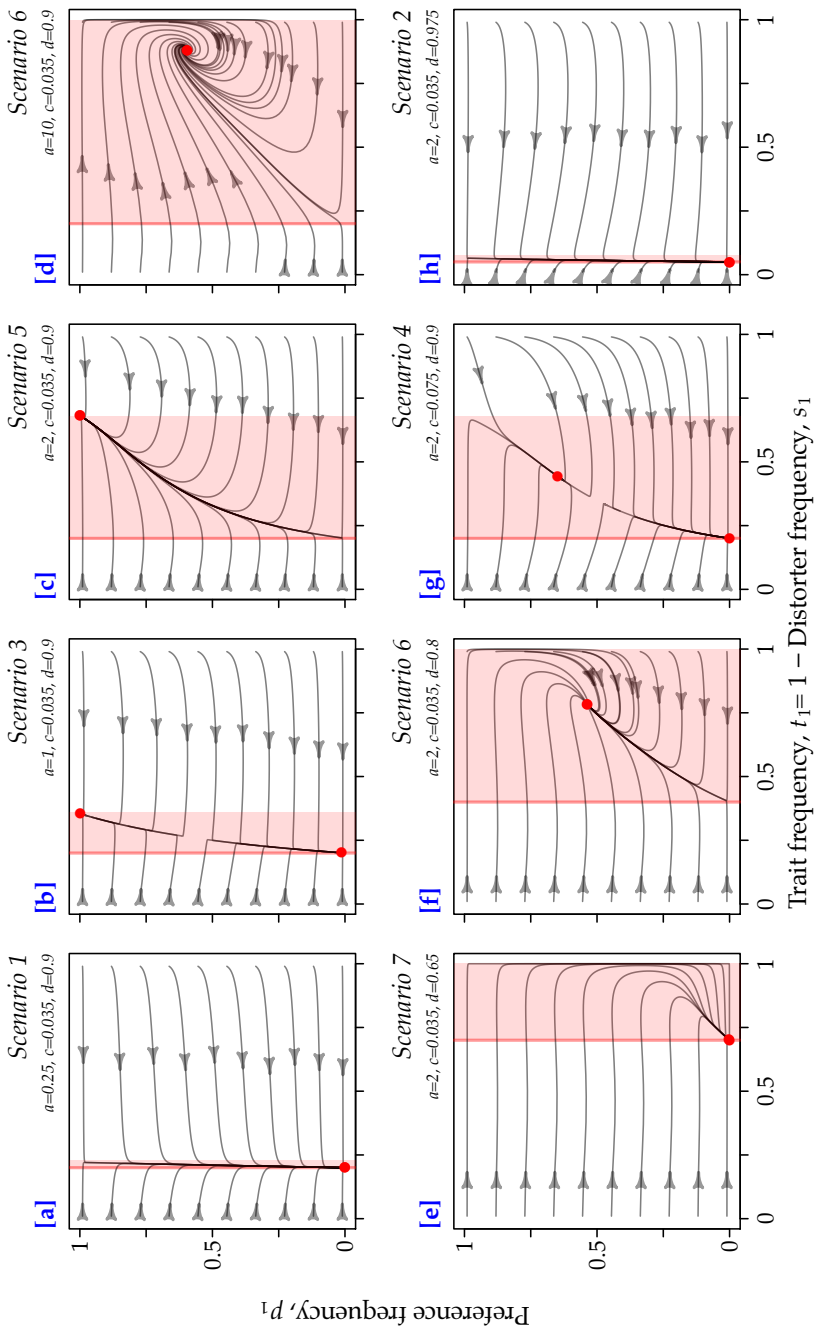

Figure 2.4. Joint evolution of distorter s_1 and preference p_1 alleles for eight different parameter combinations (also see (a–g) in Fig. 2.2), corresponding to scenarios 1–7 summarized in Table 2.2. The upper row shows evolutionary dynamics for four increasing levels of preference strength a, keeping distorter strength ($d = 0.9$) and preference costs ($c_p = 0.035$) constant. The lower row depicts evolutionary dynamics for four increasing levels of distorter strength d, keeping preference strength ($a = 2$) and preference costs ($c_p = 0.035$) constant (with the exception of (g), where $c_p = 0.075$). The red vertical line indicates the distorter equilibrium in the absence of preference $\hat{s}_{p=0}$. The red shaded area denotes the feasible distorter range. The red dots correspond to the end point of each evolutionary trajectory. Remaining parameter values: $c_t = 0$, $h_p = 0.5$, $h_a = 0$.

Table 2.2. Overview over the seven possible scenarios, corresponding to four qualitatively different evolutionary outcomes. The second column schematically illustrates the relative position of the feasible distorter range (red coloured bars) and the preference favouring range (grey colored bars).

	Condition	Schematic illustration	Evolutionary outcome P_1	Evolutionary outcome S_1	Parameter range a	d	c_p
1	$[s^-, s^+] = \varnothing$		$\hat{p}_1 = 0$	$\hat{s}_{p=0}$			
2	$\hat{s}_{p=1} < s^-$		$\hat{p}_1 = 0$	$\hat{s}_{p=0}$			
3	$\hat{s}_{p=0} < s^- < \hat{s}_{p=1} < s^+$		$\hat{p}_1 = 0$ or $\hat{p}_1 = 1$	$\hat{s}_{p=0}$ or $\hat{s}_{p=1}$			
4	$\hat{s}_{p=0} < s^- < s^+ < \hat{s}_{p=1}$		$\hat{p}_1 = 0$ or $0 < \hat{p}_1 < 1$	$\hat{s}_{p=0}$ or s^+			
5	$s^- < \hat{s}_{p=0} < \hat{s}_{p=1} < s^+$		$\hat{p}_1 = 1$	$\hat{s}_{p=1}$			
6	$s^- < \hat{s}_{p=0} < s^+ < \hat{s}_{p=1}$		$0 < \hat{p}_1 < 1$	s^+			
7	$s^+ < \hat{s}_{p=0}$		$\hat{p}_1 = 0$	$\hat{s}_{p=0}$			

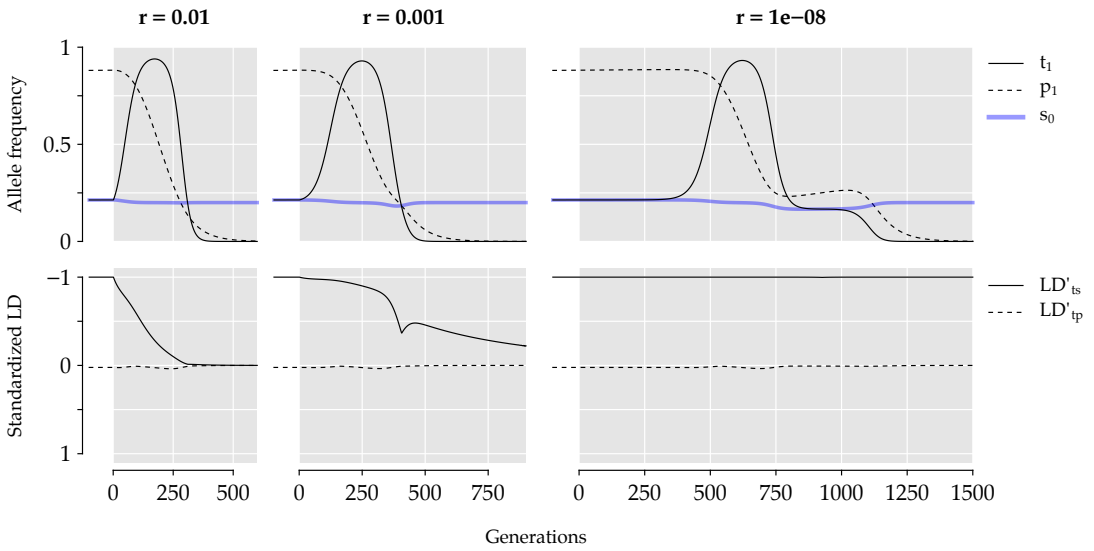

Figure 2.5. Disappearance of female preference for fair Mendelian segregation as a result of recombination between the trait and the distorter locus. Three different levels of recombination between trait and distorter are shown: $r_{TS} = [10^{-2}, 10^{-3}, 10^{-8}]$. Upper panels show allele frequencies (trait t_1, preference p_1 and non-driving allele s_0) over time. The lower panels depict standardized linkage disequilibria (LD'_{ts}, LD'_{tp}). At first, allele frequencies are fully linked and at equilibrium. At generation 0 (grey shaded area), recombination between distorter and trait is introduced. The resulting dynamics strongly resemble the case without a distorter (Fig. 1C), the costly preference ceases within a short number of generations (even at marginal recombination rates). Remaining parameter values: $a = 0.6$, $h_p = 0.4$, $h_a = 0.5$, $c_p = 0.03$, $c_t = 0.25$, $d = 0.9$.

Three-Locus Dynamics

Until now, we have assumed full linkage between the trait and the distorter locus. Hence, we considered a two-locus system where the distorter allele is a direct target of female mate choice. While this assumption may be realistic for some distorter systems (Williams and Lenington, 1993), potential male signals may be more loosely coupled to the distorter in others. As in the case of stalk-eyed flies, females will typically base their choice on a trait that is more loosely coupled to the distorter. We thus need to consider the evolutionary dynamics at all three loci. Analyzing the full complexity of the three-locus model is a daunting task. We therefore restrict our analysis to a simpler question: How do the results of the previous section change if we introduce a low level of recombination between the trait locus T and the distorter locus S (i.e. r_{TS})? To this end, we started at the equilibrium in full linkage ($LD'_{ts} = -1$) and con-

sidered the subsequent evolution of the system for a small but positive value of r_{TS}. Fig. 2.5 illustrates that preference alleles disappear from the population already at minimal levels of recombination. Recombination will produce an increasing number of T_1S_1 haplotypes that can spread —depending on recombination rates— relatively quickly through drive and sexual selection (hence the initial increase in t_1). Because choosy females increasingly mate with distorter carrying males (with detrimental consequences for their offspring), female choice will no longer confer fitness benefits. As a result, the costly mate choice allele P_1 disappears. This conclusion is representative for the whole parameter space explored in this study. We repeated the simulations of Fig. 2.2, starting with trait and distorter alleles in full linkage ($LD'_{ts} = -1$), again introducing a recombination rate $r_{TS} = 0.001$. The costly preference allele went to extinction in all simulation runs. Hence, the successful evolution of the costly preference breaks down even at marginal recombination rates between the male trait and the distorter.

2.4 Discussion

We have demonstrated that female choice for distorter-free males can spread and persist in a population even if mate choice is associated with considerable direct fitness costs. This is in contrast to classical models of sexual selection where preference costs typically result in the loss of female preference (Kuijper et al., 2012). Two key components of the distorter enable spread and maintenance of the costly female choice allele. The spread is a consequence of the large benefits associated with avoiding carriers of distorter alleles. The maintenance results from the fact that segregation distortion helps preserve male trait variation despite directional sexual selection. The balance of gene-level selection in favour and individual-level selection against the distorter alleles keeps allele frequencies at the distorter locus in a firm polymorphic state, thus avoiding the lek paradox that often hampers the maintenance of costly mate choice. Akin to previous resolutions (Kotiaho et al., 2008), the present model proposes a mechanism (distortion) that maintains trait variation in the face of directional sexual selection. Our model also identified several factors that limit the evolution of the costly preference allele. Interestingly, we find that preference evolution is limited if the distorter is very strong or if the preference allele induces strong preferences. In the latter case, the lek paradox prevails. Moreover, we show that the costly preference can only spread in the presence of a signal that reliably indicates a male's distorter genotype. Accordingly,

already the smallest degree of recombination between a male signal and the distorter will result in the disappearance of the costly preference.

Our findings are consistent with the few previous models addressing mate choice evolution in the presence of distorters, all focusing on different types of sex-linked distorters (Lande and Wilkinson, 1999; Reinhold et al., 1999; Randerson et al., 2000). In the case of sex-linked distortion, choice benefits stem from the fact that mating with a distorter-free partner will result in offspring of even sex ratio. Since the sex ratio of populations harbouring sex-linked distorters is strongly biased, producing offspring of the rarer sex convey a selective advantage as individuals of the rarer sex have a higher reproductive value (Pen and Weissing, 2001). The conclusions are similar to the ones presented here: cost-free (Lande and Wilkinson, 1999) and costly (Randerson et al., 2000) mate choice for distorter/male-killer-free mates can stably persist. Mate choice for drive suppression, on the other hand, seems not beneficial (Reinhold et al., 1999). Despite these similarities, there may be quantitative differences between autosomal and sex-linked distorters. In sex-linked distorters, relatively weak levels of sexual selection appear sufficient to drive the distorter to extinction (Lande and Wilkinson, 1999; Randerson et al., 2000). With the autosomal distorter considered here, strong sexual selection is needed to oppose distortion, allowing for a larger range of favourable conditions to the maintenance of a costly choice.

Reliable indicators of distortion Preference benefits of female choice are only guaranteed if the male trait is a reliable indicator of the genetic status at the distorter locus. In line with Lande and Wilkinson (1999), we found that already small recombination rates between trait and distorter inhibit the spread of the choice allele as they erode the reliability of the signal and hence benefits of choice. Given this restrictive prerequisite, one may conclude that our model can explain the presence of a costly preference for distorter-free mates in only few real-world systems. However, we see two scenarios in which the model can be relevant. Full linkage between a sexually selected trait and a distorter is possible if recombination between the loci is suppressed or the distorter itself is the signal (i.e. the signal is a pleiotropic effect of the distorter). Both scenarios may be relevant at different stages of mate choice.

Precopulatory stage In the case of precopulatory choice, a scenario where the distorter itself is the target of female mate choice seems unlikely. In fact, distorters typically have no or little effects on the external phenotype (Burt and Trivers, 2006) that may serve as signals for precopulatory mate choice. Suppression of recombination between the distorter and a trait 'recognizable' to females will thus be required.

Interestingly, suppression of recombination is an essential part of distorter systems as the effects of segregation distortion hinge upon the interaction of several genes (but also see van Boven and Weissing (2000)). In fact, recombination has been proposed as a way for an organism to avoid selfish action of groups of linked genes by decoupling possible alliances (Leigh, 1971). So what makes a distorter effective in the first place, suppression of recombination, may render them at the same time vulnerable to negative sexual selection. Through the lack of recombination, there is a chance that the distorter will be bound to a gene with phenotypic effects recognizable to females, thereby allowing mate choice against it. The *t* haplotype in house mice, for example, consists of about 300 genes linked to each other through four chromosomal inversions (Burt and Trivers, 2006). Among these genes are several major histocompatibility complex (MHC) loci, that have been proposed as signals mediating mate choice (Milinski, 2006). In a study on a wild house mouse population, Lindholm et al. (2013) have shown that *t* haplotypes were associated with a unique and exclusive MHC allele. Indeed, there is evidence for precopulatory mate choice in the *t* haplotype system, although the role of MHC remains controversial (Lenington et al., 1988). Overall, there is only limited evidence for precopulatory mating preferences in connection with distorters (Wedell, 2013). The requirement of a signal accidentally trapped in the distorter's linkage group may explain why it is so rare.

Postcopulatory stage Females can also avoid fertilization by distorter-carrying males at the postcopulatory stage. The mechanisms underlying segregation distortion typically lead to lower sperm number and/or lower sperm quality. As a result, distorter-carrying males are often compromised in their sperm competitive ability (Price and Wedell, 2008). Females may capitalize on this fact by mating with multiple males (polyandry), thereby avoiding fertilization by distorter-males (Haig and Bergstrom, 1995). This is interesting in the context of the model presented here, because the phenotype causing non-random mating, reduced sperm competitiveness, is a pleiotropic effect of the distorter itself. Hence, no suppression of recombination between a signal and the distorter is needed for the evolution of polyandry. This may explain why empirical evidence for polyandry as a female counterstrategy against distorters is far more abundant than precopulatory choice. Our model suggests that polyandry might be an evolutionarily stable mating strategy, even if it is more costly to females than monandry. However, choice here is based on a fixed relative preference, i.e. the strength of preference is independent of distorter frequency, whereas in the context of polyandry, sperm competition only matters if both male types are present in the sample of males

a female mates with. The effectiveness of polyandry will hence be a function of distorter frequency. A 'best-of-N' mechanism, where females choose a male of a given male subsample (Seger, 1985), may be a more adequate depiction of polyandry. In a recent study, Holman et al. (2015) investigated this scenario in the context of a sex-linked distorter. They found that the evolution of costly polyandry can indeed evolve in circumstances where the sex-distorter is associated with additional organismal fitness costs. Further studies are required to investigate how such an alteration in the mate choice mechanism affects the evolutionary outcomes in autosomal distortion systems.

Maintaining Distorter Alleles Our model demonstrates that a costly mate choice can only successfully evolve if distorter frequencies are kept at intermediate frequencies, where the preference allele is selectively favoured. The successful spread of the preference allele is hence the result of a delicate balancing act. Any selective force that pushes distorter frequencies to one or the other extreme of the distorter frequency spectrum will limit the evolution of costly choice. Accordingly, we found that very strong or weak levels of segregation distortion hamper the spread of the preference allele. Similarly, a costly mate choice can only be maintained efficiently if the preference is of intermediate strength. If directional sexual selection is strong, it may override the distorter's capacity to create new male trait variance. In this case, the lek paradox prevails. Once choosy females have successfully removed most distorters from the population, i.e. male variation has expired, preference costs again start to outweigh preference benefits, just as in our original scenario without a distorter (where already marginal levels of preference are sufficient to run into that problem, see Fig. 2.1b). Preference frequency will then stabilize at a lower level that allows for enough male trait variation to keep benefits and costs of choice in balance (see Fig.2.4d,f). In other words, costly mate choice for Mendelian segregation will only escape the lek problem in a given spectrum of preference strength. At the lower end of the spectrum, choice is not effective and benefits of choice are hence limited. At the upper end of the spectrum, sexual selection is —once more— too strong to maintain male trait variation. It is unclear if the levels of preference strength needed for this second effect are biologically relevant. However, the question whether there is an optimal level of preference strength is an interesting theoretical question in itself, especially considering the non-straightforward relationship between preference strength and equilibrium preference frequency.

So far, we have largely focused on the distorter's influence on the sexual selection process. However, we can also ask how costly female choice affects distorter

dynamics. Accounting for distorter frequencies in wild populations is a long standing focus of evolutionary theory (Burt and Trivers, 2006). Depending on its strength, female mate choice may be an important determinant of distorter frequency (e.g. Manser et al. (2011)). Akin to molecular suppressors of distortion proposed elsewhere (Charlesworth and Hartl, 1978; Hiraizumi and Thomas, 1984), female mate choice may be regarded as a suppressor of distortion at a behavioural level. By undermining the spread of the selfish distorter, female choice may help to maintain harmony at the genomic level. However, our current model suggests that this mechanism will only be successful to a certain degree, at least as long as female choice is costly and drift effects are negligible. The scenario where the lek paradox prevails as a consequence of strong directional sexual selection (scenario 2) makes clear that female choice will never completely remove the distorter. As soon as mate choice is effective in removing distorter alleles, benefits of choice fade, allowing the distorter back in. Intriguingly, this second order lek paradox may provide an explanation for another well-established paradox, known as the low *t* frequency paradox in house mice. In the *t* haplotype system in house mice, *t* frequencies in wild populations are usually at low (lower than expected from distortion and lethality only) but stable levels (Ardlie, 1998; van Boven and Weissing, 1999). Costly female choice may explain why *t* frequencies are lower than expected, yet stably prevail in populations.

A general mechanism for the evolution of costly mate choice? The presented model demonstrates that segregation distorters can greatly facilitate the evolution of female choice, even if such a choice is associated with substantial fitness costs. We can only speculate about the importance of distorter systems for the evolution of female choice in general. Selfish genetic elements are considered a ubiquitous feature of life (Burt and Trivers, 2006). However, the abundance of autosomal distorter systems considered here, particularly among animals, is largely unknown. The covert action of distorters make detection and identification inherently difficult. It is not surprising that the best known distorter systems were both found in two of the best-studied model organisms (*t* haplotype in the house mouse and *Segregation Distorter* in *Drosophila*). Deviations from Mendelian inheritance are occasionally reported in other species, but the causes of such biased inheritance is often unknown (Burt and Trivers, 2006). In both known cases, segregation distortion is relatively effective ($d \approx 0.9$). It is not known whether this feature is representative of distorter systems in general or whether it is the result of a detection bias (as weaker distorters are more difficult to discover). Our model suggests that a weak distorter's capacity to promote female mate

choice is reduced, because weak distortion easily results in distorter equilibria outside the preference favouring range (scenarios 6 and 7). However, if not only distortion is weaker, but also its selective effects on the organism (here, distorters result in male sterility or homozygote lethality), distorter equilibria may well shift back into the preference favourable range. In any case, the present model shows the action of distorters, usually hidden from sight, may play important role in driving the evolution of costly female choice, both at a pre- and postcopulatory stage.

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Chapter 3

Female House Mice avoid Fertilization by *t* Haplotype Incompatible Males in a Mate Choice Experiment

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Abstract

The *t* haplotype in house mice is a well-known selfish genetic element with detrimental, non-additive fitness consequences to its carriers: recessive lethal mutations cause *t*/*t* homozygotes to perish *in utero*. Given the severe genetic incompatibility imposed by the *t* haplotype, we predict females to avoid fertilization by *t* haplotype incompatible males. Indeed, some of the strongest evidence for compatibility mate choice is related to the *t* haplotype in house mice. However, all previous evidence for compatibility mate choice in this system is based on olfactory preference. It is so far unknown how general these preferences are and whether they are relevant in an actual mating context. Here, we assess female compatibility mate choice related to *t* haplotypes in a setting that —for the first time— allowed females to directly interact and mate with males. This approach enabled us to analyze female behaviour during the testing period, and the resulting paternity success and fitness consequences of a given choice. We show that genetic incompatibilities arising from the *t* haplotype had severe indirect fitness consequences and *t* females avoided fertilization by *t* incompatible males. The results are inconclusive whether this avoidance of *t* fertilization by *t* females was caused by pre- or postcopulatory processes.

Keywords: Sexual Selection, Mate Choice, Genetic Compatibility, *t* Haplotype, Selfish Genetic Elements, Polyandry

3.1 Introduction

Female mate choice is recognized as a powerful evolutionary process, potentially explaining the origin of extravagant male ornaments that remain puzzling under the concept of natural selection. However, the question of why females choose mates at all, especially in the absence of direct fitness benefits (indirect selection), remains a much debated topic in sexual selection research. Indirect selection on female mate preference occurs if the preference trait is genetically correlated to a trait under direct selection. A variety of mechanisms have been proposed about how such a correlation can arise, ranging from Fisher's runaway hypothesis (Fisher, 1930), where a male display trait becomes genetically correlated with the female preference for that trait, to indicator or 'good genes' models where the male display trait is used as a signal of male genetic quality (Andersson, 1994). Selection should also favor female preference for partners that produce offspring with the most adaptive gene combinations. This concept of mate choice for 'compatible genes' —originally advanced by Trivers (1972)— has only recently received attention in empirical studies (Mays Jr and Hill, 2004). Under this paradigm, the best mate for a given female does not only depend on the male's genotype (such as in 'good genes' models), but also on her own genotype (Tregenza and Wedell, 2000; Zeh and Zeh, 2003, 1996, 1997). In contrast to 'good genes' hypotheses that assume additive gene action (such that an optimal choice for a female is independent of her own genotype), mate choice for compatibility is based on non-additive genetic effects such as dominance or epistasis (Kotiaho et al., 2008). On a between-species-level, mate choice for compatibility, i.e. preference for conspecific partners, is well documented and is important in the process of sympatric speciation (Butlin and Ritchie, 1989). On the within-population level, however, the importance of genetic compatibility remains unclear. Non-additive effects are likely to be complex if many genes are involved. Selection for genetic compatibility is therefore likely to be constrained to specific genetic systems with potentially large fitness effects (Puurtinen et al., 2005).

Selfish genetic elements and genetic incompatibility. A promising class of genetic systems to drive the evolution of mate choice for genetic compatibility are selfish genetic elements (SGEs henceforth, Zeh and Zeh (1996); Tregenza and Wedell (2000)). SGEs are broadly defined as stretches of DNA that promote their own transmission at the expense of rival genes (Burt and Trivers, 2006). Most known SGEs are associated with substantial fitness costs to their carriers (Burt and Trivers, 2006).

Despite these detrimental effects on carrier fitness, SGEs often stably persist in populations as a result of their systematic transmission advantage. The stable presence of a SGE may select for female mating strategies to avoid substantial, SGE-related fitness losses. Females could avoid carrier males at a pre- or postcopulatory stage. There is only limited evidence for discrimination against SGE-carriers prior to mating. In stalk-eyed flies, females prefer to mate with males with long eye-stalks. In populations that harbour a sex-ratio distorter, it has been shown that long male eye-stalks indicate a genetic suppressor of drive (Cotton et al., 2014). A preference for long eye-stalks will hence ensure that females produce both sons and daughters (Wilkinson et al., 1998). Because SGEs often target male spermatogenesis to achieve transmission advantage (termed drive), male SGE-carriers are often compromised in their sperm competitiveness (Price and Wedell, 2008). Females may capitalize on this link and avoid SGE-fertilization by mating with multiple males (polyandry) thereby enhancing the importance of sperm competition (Haig and Bergstrom, 1995). Empirical evidence for the importance of such postcopulatory SGE-avoidance is numerous, especially in insect species (see Wedell (2013) for a recent review). For example, in sex-ratio drive systems of *Drosophila simulans* and *Drosophila pseudoobscura*, distorters were shown to considerably reduce competitive ability of gametes (Atlan et al., 2004; Price et al., 2008a). In the latter case, female flies were even found to evolve higher remating rates in the presence of the distorter (Price et al., 2008b).

In the previous examples, all females are susceptible to the negative fitness effects of the SGE and hence all females should avoid carriers. They can therefore not be considered cases of choice for compatibility. However, SGE-related fitness costs are often non-additive, hence causing incompatibilites between maternal and paternal genotypes (Zeh and Zeh, 1996). As a consequence, the fitness consequences of a given female mating decision is not only a function of her partner's genotype, but also of her own. In *Drosophila paulistorum* flies, both males and females mate assortatively based on the intracellular bacterium *Wolbachia* (Miller et al., 2010), helping them to avoid *Wolbachia*-induced embryo mortality and male sterility. Such *Wolbachia*-related assortative mating has also been reported in the spider mites *Tetranychus urticae*, where uninfected females avoided incompatible infected males in mate choice tests (Vala et al., 2004).

The t haplotype. Some of the strongest evidence for compatibility mate choice is related to the *t* haplotype system in house mice. The *t* haplotype in house mice is a classical example of a SGE. It consists of a whole set of genes occupying about one third

of mouse chromosome 17 and is protected from recombination by a large inversion system (Silver, 1993). The *t* haplotype is thought to have existed for more than 3 million years (Burt and Trivers, 2006) and occurs in populations of all four house mouse subspecies (Silver, 1993). It has the properties that make it a promising candidate for compatibility mate choice. First, the *t* haplotype is associated with substantial non-additive fitness costs. Most *t* haplotypes carry recessive embryonic lethal mutations, causing t/t homozygotes to perish *in utero* (Klein et al., 1984). $+/t$ heterozygotes, on the other hand, are fully viable. Second, several genes within the complex ensure that this genetic entity is passed in a non–Mendelian manner from one generation to the next (drive). As a result, heterozygote $+/t$ males typically transmit their *t* gametes to 90% of their offspring (Lyon, 2003; Lindholm et al., 2013). $+/t$ females show normal Mendelian segregation. The resulting genetic incompatibilities are severe. A $+/t$ female that mates with a $+/t$ male is expected to lose up to half (depending on levels of drive) of her offspring from *t* lethal effects. Indeed, Lindholm et al. (2013) recently reported a litter size reduction of 40% at birth in controlled laboratory crosses. Hence, selective pressures on $+/t$ heterozygote females to avoid *t* fertilization are substantial. Lenington and colleagues showed a consistent odor preference of $+/t$ females towards $+/+$ males in a series of experiments (see Lenington (1991) for a review). Results for $+/+$ females were not as clear: in some studies, they exhibited the same preference for $+/+$ males (Lenington, 1983; Lenington and Egid, 1985), whereas in others preferences were not found (Coopersmith and Lenington, 1992; Williams and Lenington, 1993). It remains unclear whether these olfactory preferences reflect actual mating preferences and whether they are generalizable to different *t* haplotype variants (16 *t* haplotype variants have been described so far, Klein et al. (1984)). The role of polyandry and sperm competition in the *t* haplotype system is largely unknown. High multiple paternity rates in wild populations suggest that sperm competition plays an important role in house mice (Dean et al., 2006; Firman and Simmons, 2008c; Manser et al., 2011). However, there is only very limited data on sperm competitiveness of *t*-carrying males. In the three previous studies looking at fertilization success of $+/t$ males when competing with $+/+$ males, paternity shares of $+/t$ males ranged between 0.17 and 0.36, but were based on very limited sample sizes (Ardlie and Silver, 1996; Carroll et al., 2004; Olds-Clarke and Peitz, 1986).

In the present study, we aimed to experimentally test female mate choice in relation to the *t* haplotype using an elaborate choice device. The device allowed females to freely associate and mate with either or both males of different genetic background

(+/t and +/+, termed t and w for wildtype hereafter) without interference of direct male-male competition. Paternity analyses of the resulting offspring as well as behavioral data during the experiment allowed us to address the following three questions. (1) What are the fitness consequences of female mating decisions? (2) Do t females avoid fertilization of incompatible t males? (3) Are potential fertilization biases caused by pre- or postcopulatory processes?

3.2 Methods

Animals

The animals used for mating preference tests were all descendants from a wild house mouse *(Mus musculus domesticus)* population outside Zurich, Switzerland (see König and Lindholm (2012) for details). They were kept under standard conditions (22–25 °C, 40-50% humidity, 14:10h light:dark cycle starting at 7:30) with *ad libitum* food (laboratory diet for mice and rats, no. 3804 and 3336, Provimi Kliba SA, Kaiseraugst, Switzerland), water, nesting material, and standard animal bedding (Lignocel Hygienic Animal Bedding, JRS). Altogether, 65 females and 45 males were used in the experiments. All males and females were typed at the *Hba-ps4* locus —a marker containing a 16-bp t haplotype specific insertion (Hammer et al., 1989)— to determine genotype at the t locus. The level of segregation distortion for the laboratory population has been estimated previously and is 90% (Lindholm et al., 2013). No females, but all males had mating experience prior to the experiment.

Choice Device

An elaborate choice device developed in our group (Rüsch, 2002) was used to assess female mating strategy (see Fig. 5.1). The device consisted of three Macrolon II cages (A–C) connected with tunnels separated by doors. Only the female was allowed free access to all three cages (A–C). Males on the other hand were confined to their own, outer cages (A and C). To achieve this, all individuals used in the experiment were tagged individually with transponders (glastag micro read only, article no. 860-0220, IQ Automation GmbH, Eching, Germany). Transponders were recognized by specific readers (1–4, easy key Standalone module, article no. A402-0031, IQ Automation GmbH, Eching, Germany) over antennae that were wound manually around the tun-

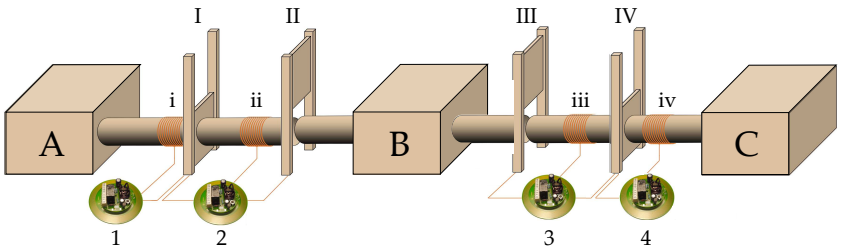

Figure 3.1. Scheme of choice device with the three cages (A–C), four doors (I–IV), four antennae (i–iv) and their corresponding readers (1–4). Males were confined to cages A and C, respectively. The female had access to all three cages.

nels at positions (i)–(iv). Each of these readers was further connected to specific doors (readers 1 and 4 to doors I and IV, respectively, and readers 2 and 3 to doors I+II and III+IV, respectively). Readers were specifically programmed to open/close the associated doors after the recognition of transponder numbers of choice. Free female movement (a) and male confinement to their own cage (b) were guaranteed by the following settings.

(a) In its initial state, inner doors (II and III) were open and outer doors (I and IV) were closed and the female was placed in her home cage (B). Inner antennae (ii and iii) only reacted to female transponders. When the female passed by an inner antenna, for example (ii), her transponder was read by the antenna device (2) and the door status was switched (the inner door II was closed and the outer door I was opened). The female could hence freely access a male cage (A in this example). The same process applied for the opposite direction and allowed the female to return to the middle cage (B).

(b) Outer doors were only open when a female was visiting, entering or leaving. To prevent male escape, outer antenna (i and iv) were programmed specifically on male transponders, causing an immediate closing of the outer door as soon as the male was read by its antenna. The door remained closed as long as the male was within the reach of this antenna.

Experimental Procedure

Females of both genetic backgrounds (*t* and *w*) were given the choice between a *t* male and a *w* male. Weight difference between males never exceeded 2 g and no combination of males was used twice. Most males were used several times with different females. None of the individuals used in the same test were siblings. As we had five choice devices at our disposal, up to five tests could be run at the same time. All cages (A–C) were equipped with standard animal bedding, nesting material, and *ad libitum* food and water. Choice tests were divided into two phases.

Priming. To habituate females to the device and to initiate estrous, females were first put into the device *without* males in the side cages for 1 day. The side cages had previously housed the test males for one day and thus contained soiled bedding. Allocation of genotypes to positions A and C was randomized. Males were removed just before the start of the trial. In the presence of male urine, females initiate an estrous cycle (Bronson, 1979). Wild house mice quickly learned to open doors by moving through the fields of the antennae.

Choice test. After the priming phase, males were put back into their cages and sides were swapped systematically. Male tests lasted 5–12 days so as to encompass at least one full estrous cycle. An estrous cycle typically lasts four days, with estrous occurring during one night (Bronson, 1979). We did not use invasive assessments to determine estrous (vaginal smears) to minimize disturbance and repeated handling of females.

At the end of the preference test, cage B was removed and used as the respective female's home cage, again to minimize handling of females. Females were daily checked for birth of a litter and offspring were counted when present. Tissue sampling of the pups for paternity analysis was done by an ear punch at the age of 13 days or when pups were found dead.

This experiment was approved by the Veterinary Office Zürich, Switzerland (permit 97/2009).

Sample sizes. In total, we tested 31 *t* and 34 *w* females (see Table 3.1 for an overview). Some females were tested again if previous tests did not result in offspring. Overall, we conducted 83 mate choice tests on 65 females.

	t females	*w* females	total
females tested	31	34	65
test repeats	14	4	18
total number of tests	45	38	83
females producing offspring	19	15	34
behavioural data available	16	20	36
offspring and behavioural data	9	9	18

Table 3.1. Overview of choice test sample sizes.

Paternity Analysis

All offspring produced in the experiment, their mothers and the two candidate fathers of the relevant trials were genotyped at 5 neutral microsatellite loci in a single multiplex reaction (average $H_e = 0.75$, average number of alleles per locus was 6.2). If variation at these markers was insufficient to unambiguously assign paternity in any trial, an additional 6 neutral microsatellite loci were amplified in a second multiplex reaction. Paternity analyses were performed using maximum likelihood as implemented in Cervus 3.0 (T. Marshall, Field Genetics Ltd.). Paternity assignments were made at a confidence level of 95%.

Statistical Analysis

Litter sizes Litter sizes were analyzed as a function of female genotype (*t* and *w*), their genetic fathers (three categories: *t* male, *w* male, and multiple paternity) and their interaction in a generalized linear model using an exponential link function and a Poisson error distribution.

Fertilization bias The results of the paternity analyses were used to estimate fertilization bias, i.e. proportion of offspring sired by the *t* male given female genotype i, $F_i \in [0,1]$. Any systematic deviation from no preference ($F_i = 0.5$) can be the result of pre- or postcopulatory mate choice. Hence, we were interested in two pieces

of information: (a) The *mean* prediction $E[\hat{F}_i]$ indicates whether there is a systematic deviation from no choice expectations ($H_0 : F_i = 0.5$). This information will not help to distinguish between pre- and postcopulatory choice processes. For this, we need to estimate (b) *variance* $V[\hat{F}_i]$ (see below for more details). However, a binomial generalized linear model (GLM) —the standard approach for this type of data— will not estimate the variance (because binomial errors directly follow from binomial parameters p and n). Therefore, we ran three custom null models using computer simulations. Each null model assumed a specific fertilization probability (see below), for which we derived the expected probability distribution of F_i (see Fig. 3.3B). From these expected distributions, we drew 10^5 values based on our sample sizes ($n_t = 19$, $n_w = 15$), giving us a population of realized mean $E[F_i]$ and variance $V[F_i]$ expectations. These expectations were compared to observed mean $E[\hat{F}_i]$ and variance $V[\hat{F}_i]$. Here is a short description of the three null models.

- *Null model 1: Precopulatory process only; no choice.* In this null model, no polyandry occurs and females have no preference for either male genotype. All offspring will hence be sired completely by one of the two males, F_i for an individual female represents a Bernoulli trial with probability $p = 0.5$, i.e. $F_i \sim Bernoulli(p = 0.5)$.

- *Null model 2: Postcopulatory process only; no choice.* Here, all females mate with both males at equal frequency, but t males do not suffer from sperm competition disadvantages. F_i hence follows a binomial distribution with $F_i \sim Binom(p_i, n)$, where n denotes litter size and p_i the fertilization probability of a t male in a female of genotype i. In w females, both males have equal chances of fertilization, i.e. $p_w = 0.5$. In t females however, F_t will be reduced as a proportion of sired zygotes will die during embryogenesis due to t/t recessive lethal effects. This proportion is a function of segregation distortion τ. For the mice used in this study, $\tau = 0.9$ (Lindholm et al., 2013). We hence have $p_t = \frac{1-\tau/2}{2-\tau/2}$, as $\frac{\tau}{2}$ t sired embryos perish *in utero*. Observed average litter size was $E[\hat{n}] = 6.11$ and followed a Poisson distribution (mean-to-variance ratio $\frac{E[\hat{n}]}{V[\hat{n}]} = 1.2$). Thus, litter sizes n were drawn from a Poisson distribution with $\lambda = 6.11$.

- *Null model 3: Postcopulatory processes only; t sperm disadvantage; no choice.* In the third null model, females again mate invariably with both males, so again $F_i \sim Binom(p_i, n)$. This time, however, t males do worse in sperm competition. If

sperm number is an important determinant of a male's sperm competitive ability, t males are expected to be poor sperm competitors as a substantial fraction $1 - \frac{1}{2\tau}$ of their gametes are rendered dysfunctional by the distorter. Based on the difference in functional sperm number only, we expect $p_w = \frac{1}{1+2\tau}$ and $p_t = \frac{2-\tau}{2+3\tau}$ (Manser et al., 2011).

Due to the limited number of animals available, some males were used repeatedly across tests (on average, males were used 1.7 ± 0.9 times). Note that we had no repeated measures of F_i for females, as females were only tested again if the previous trial did not result in offspring. Our custom model approach did not allow us to account for the repeated use of males in our experiment. To account for this non-independence, we additionally analyzed fertilization biases F_i as a function of female genotype (t or w) in a generalized linear mixed effects model (GLMM) with a logit link function and a binomial error distribution, using t and w male identity separately as random effect variables.

Behavioural preference. Every time a transpondered animal was read by an antenna in the choice device system, a data string containing information about transponder number, time and origin was sent to a computer and logged with a specific program (Advanced Serial Data Logger, AGG Software, Kolchugino, Russia). The positional information was used to assess female behavioural preference. An entry into one of the male cages was scored if a female log entry of the inner antenna was followed by a record of the outer antenna. An exit was scored in case of the opposite order. With this information in hand, the duration of female visits in the t and w males cage was determined. The analysis of the visit duration was only started after the female had visited both male cages at least once. Behavioural preference of a female of genotype i was defined as

$$B_i = \frac{T_t}{T_t + T_w} \in [0, 1]$$

where T_t and T_w is the total time a female spent in the t and w male cage, respectively. Observed behavioural preferences $\hat{B}_i$ were logit transformed (Warton and Hui, 2011) and analyzed as a function of female genotype using a linear mixed effects model (with $H_0 : B_i = 0.5$). To account for the repeated use of males, t and w male identity were used as seperate random effect variables. There was no repeated measure of B_i for females.

Influence of behavioural preference on fertilization biases. In order to find out if behavioural preference B_i were predictive for t paternity shares F_i, we analyzed F_i as a function of female genotype i and behavioural preference B_i and their interaction. In contrast to the analysis above, we used an arcsine-square-root transformation of the response variable here (as F_i-values of 0 and 1 transform to undefined values $-\infty$ and ∞, respectively, when logit transformed). The full model was reduced to the minimal adequate model using likelihood-ratio tests.

Data processing, statistical analysis and computer simulations were carried out in R 3.0.0 for Mac (R Core Team, 2014).

3.3 Results

Litter sizes. 34 of 83 mate choice tests (41%) resulted in offspring. Average litter size was 6.11 ± 2.21 (mean $\pm$ sd). In t females, litter sizes were significantly lower when mating with a t male than a w male (95% CI for difference between t and w sired litters: $[1.24, 5.94]$, $z = 2.17$, $P = 0.030$; see Fig. 3.2). Multiply sired litters on the other hand were not reduced in litter sizes (95% CI for difference between w and multiply sired litters: $[-2.04, 2.83]$, $z = 0.63$, $P = 0.528$). In w females, litter sizes were also smaller when t sired, but this was not statistically significant (95% CI w vs. t sired: $[-0.76, 4.11]$, $z = 1.52$, $P = 0.129$; 95% CI w vs. multiply sired: $[-4.43, 2.55]$, $z = -0.10$, $P = 0.922$).

Fertilization biases. Figure 3.3A shows distributions of observed paternity biases F_i for t and w females. Figure 3.3B shows the expected distributions of F_i based on the three null model simulations as well as distributions of mean $E(F_i)$ and variance $V(F_i)$ predictions for the given sample sizes ($n_t = 19, n_w = 15$) resulting from 10^5 simulation runs. Table 3.2 summarizes the comparisons between the observed data and expectations of the Null model simulations. As mentioned in the Methods, mean predictions may help us understand possible deviations from no choice expectations, whereas variance predictions may help to distinguish between pre- and postcopulatory processes.

- *Mean predictions:* The observed t females' paternity bias $\hat{F}_t$ deviates significantly from 'no choice' expectations, either solely based on precopulatory (Null model 1) or postcopulatory processes (Null model 2). $\hat{F}_w$, on the other hand, do not

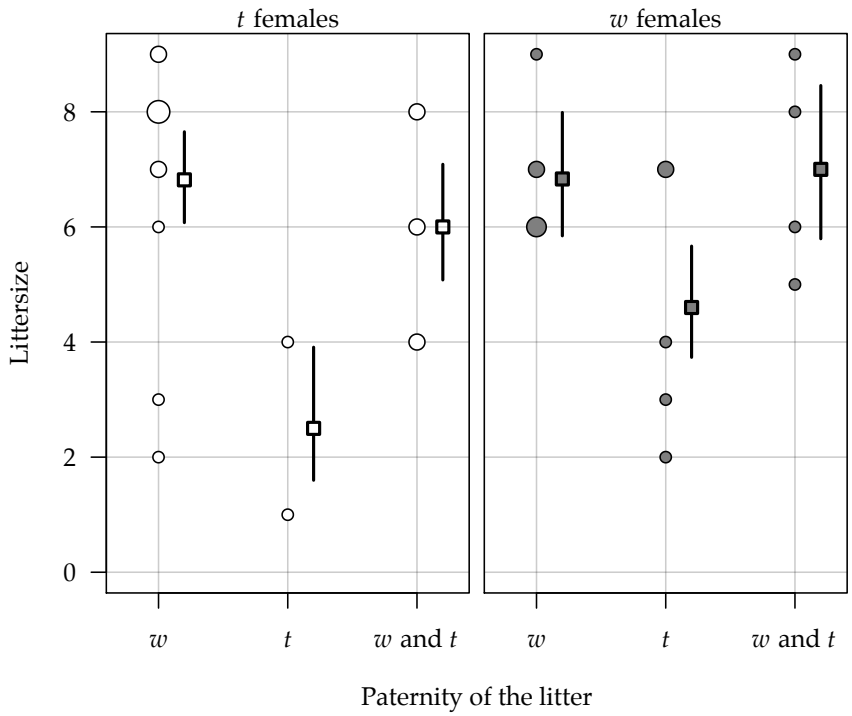

Figure 3.2. Litter sizes ± standard errors of the mean (SEM) as a function of mother genotype (two panels) and the paternity of the litter, i.e. the genotype of the genetic father of the litter (either exclusively *w*, exclusively *t*, or both). The surface of the circles are proportional to the number of observations.

deviate from 'no choice' expectations (Null models 1 and 2). This leaves Null model 3 as the only one fully compatible with mean $\hat{F}_i$ observations.

- *Variance predictions:* Predicted variance patterns strongly differ between the different Null models: Null model 1, which assumes precopulatory processes only, predicts substantially higher variances in F_i than the Null models 2 and 3 which are based on postcopulatory processes. A comparison to the observed data may therefore help us to distinguish between the two processes. In fact, none of the Null models is in line with the observed variance patterns, the observed variance lies between the low variance predictions of Null model 2 and 3 and the high variance predictions of Null model 1.

The conclusions are unaffected when we account for the repeated use of males across tests. The mixed effects model (GLMM) confirmed that F_t was significantly

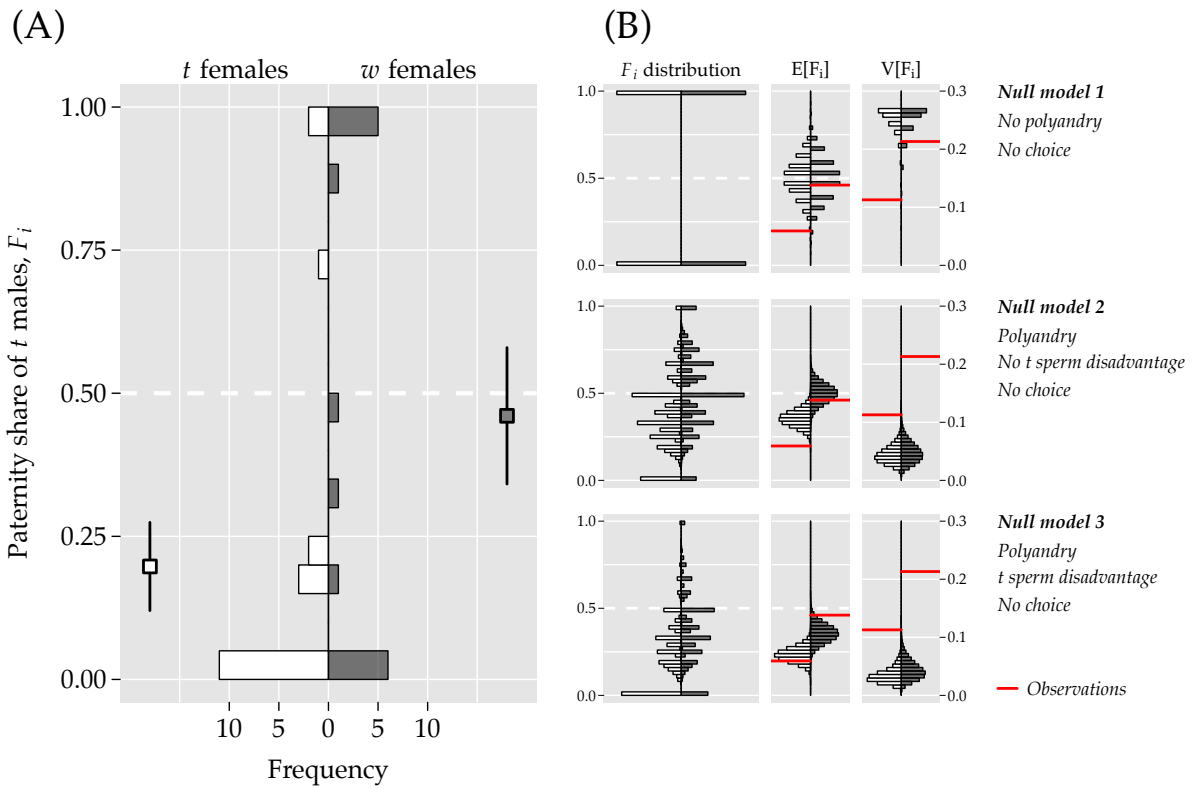

Figure 3.3. Observed (A) and expected (B) distributions of fertilization biases F_i for t (white) and w (dark-grey) females. Dashed horizontal lines represent no fertilization bias ($F_i = 0.5$). Squares in (A) depict mean observed values $\hat{F}_i \pm$ standard error of the mean (SEM). (B) Left panels show F_i distributions based on the three different null models. Center and right panels show the resulting mean $E[F_i]$ and variance $V[F_i]$ expectations for 19 t and 15 w females (based on 10^5 draws). Observed values are shown as red horizontal lines.

different from no choice expectations ($z = -2.58$, $n = 34$, $P = 0.010$). F_w, on the other hand, did not deviate from no choice expectations ($z = -0.82$, $n = 34$, $P = 0.415$). The data were not overdispersed (dispersion parameter: 0.92).

Behavioural preference. The software recording of female behaviour was available for 71 choice tests, of which data from 36 tests had to be discarded due to technical problems in the recording of the data (Table 3.1). For the remaining 35 tests, a total 6,414,744 log entries accumulated. Females visited either male cage on average 84.4 ± 44.8 (mean $\pm$ sd) times per day. Average visit duration was 9.52 ± 9.48 min. They spent 37.27 ± 17.14% of their time in a male's cage (see also Supplementary

	***t* females**		***w* females**	
	$E[F_t]$	$V[F_t]$	$E[F_w]$	$V[F_w]$
Observations	0.20	0.11	0.46	0.21
Null model 1	*Precopulatory processes only; no choice*			
	$F_t \sim Bernoulli(p = 0.5)$		$F_w \sim Bernoulli(p = 0.5)$	
	0.50	0.25	0.50	0.25
P–value	0.020	< 0.001	0.607	0.119
Null model 2	*Postcopulatory processes only; no choice*			
	$F_t \sim Binom(p_t = \frac{1-\tau/2}{2-\tau/2}, n)$		$F_w \sim Binom(p_w = \frac{1}{2}, n)$	
	0.36	0.044	0.50	0.049
P–value	0.001	< 0.001	0.489	< 0.001
Null model 3	*Postcopulatory processes only; t sperm disadvantage; no choice*			
	$F_t \sim Binom(p_t = \frac{2-\tau}{2+3\tau}, n)$		$F_w \sim Binom(p_w = \frac{1}{1+2\tau}, n)$	
	0.23	0.035	0.36	0.045
P–value	0.393	< 0.001	0.059	< 0.001

Table 3.2. Observed mean and variance of t paternity shares ($E[\hat{F}_i]$, $V[\hat{F}_i]$) in comparison to the three null model expectations.

Figures). Figure 3.4A shows behavioural preferences for the t male $\hat{B}_i$ for t and w females. According to the linear mixed effects model using a logit-transformation, the 95% confidence bands of $\hat{B}_i$ did not fall outside the no choice predictions ($B_i = 0.5$) both in t females (95% CI: $[0.10, 0.64]$) and w females (95% CI: $[0.16, 0.75]$).

Influence of behavioural preference on fertilization biases For 9 t and 9 w females, estimates for both behavioural preference B_i and genetic preference F_i were available. According to the model selection procedure using likelihood-ratio tests, behavioural preference B_i was significantly associated with t paternity shares F_i (see Fig. 3.4B, $t_{15} = 2.46$, $P = 0.026$). Female genotype, on the other hand, was removed by model selection.

3.4 Discussion

This study showed that (1) genetic incompatibilities arising from the t haplotype had severe indirect fitness consequences and (2) t females avoided fertilization by t-locus incompatible males. This is the first experimental evidence to show that t females avoid t incompatible males in an actual mating context. The results are inconclusive whether this avoidance of t fertilization by t females was caused by pre- or postcopulatory processes.

The cost of genetic incompatibility We found a severe litter size reduction of about 60% if t females mated with genetically incompatible t males rather than a compatible w male (Figure 3.2). However, this estimate is based on a very low sample size (as a consequence of free partner choice, only two females had completely t-sired offspring). In a previous experimental study on the same mouse population, litter sizes were reduced by 40% (Lindholm et al., 2013). In that study, examination of uterine scars confirmed that this reduction is a result of prenatal mortality. The difference in litter size reduction to the present study is likely a consequence of sampling error, i.e. the low sample size in the present study. w females also suffered from a slight reduction in litter size when mating with t males, but this reduction was not statistically significant ($P = 0.13$). Based on a larger sample size, Lindholm et al. (2013) found a similar, non-significant trend towards reduced t male fertility. This trend would suggest a fertility difference between t and w males, which has been reported previously (Lenington et al., 1994; Carroll et al., 2004; Ardlie and Silver, 1998). In contrast to Carroll et al. (2004), we found no indication of reduced t female fertility, both in this

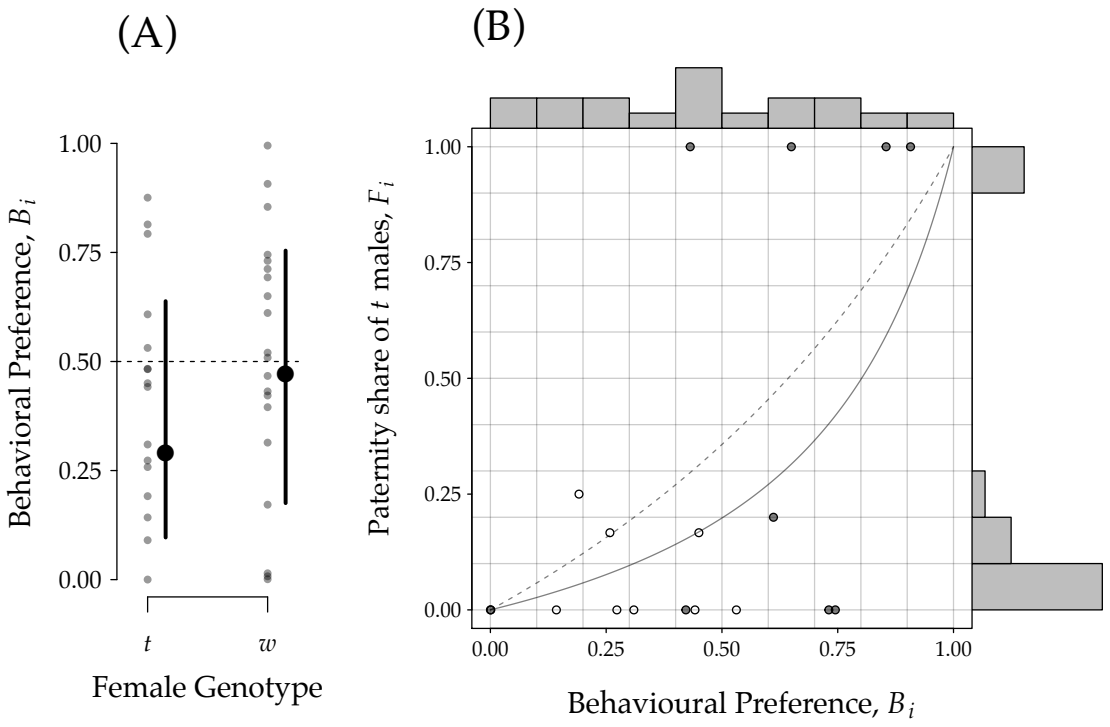

Figure 3.4. (A) Behavioral preference for t males B_i as a function of maternal genotype including mean and 95% confidence interval estimates. The dashed horizontal line depicts the null hypothesis, i.e. no choice ($B_i = 0.5$). (B) Paternity share of t males F_i as a function of behavioural preference B_i. The color of the dots represent female genotype (white: t, dark-grey: w). The histograms at the figure margins depict the distribution of the data in both x- and y-direction, illustrating that nearly uniformly distributed behavioural preference B_i translate into clear fertilization biases F_i. Dotted and solid lines show expected paternity shares for t and w females, respectively, based on Null model 3. It is assumed that a female's behavioural preference B_i is proportional to the number of matings as well as the number of competing sperm of a given male. For example, if $B_i = 0.5$, both males contribute equally to the competing sperm pool. However —according to null model 3— a proportion $1 - \frac{1}{2\tau}$ of a t male's sperm is dysfunctional.

study and Lindholm et al. (2013). Furthermore, we show that females can avoid litter losses arising from genetic incompatibility by mating with both males (polyandry): litter sizes of multiply sired litters did not differ from litters exclusively sired by w males. This result suggests that t males are poor sperm competitors (see also below). Overall, litter size results nicely outline the two possible female strategies to avoid fertilization by incompatible t males, i.e. maximize litter size: females can either avoid t males prior to mating or mate multiply and rely on sperm competition to reduce t fertiliza-

tion.

Mate choice for genetic compatibility The litter size results highlight the severe selective pressure on t females to avoid fertilization by genetically incompatible t males. Indeed, we found that t females successfully avoided t male paternity when given free choice between a t and a w male. Only 20% of all t female offspring were sired by the t male. This proportion was significantly different from no choice expectations of scenario 1 (50%) and scenario 2 (36%). There is ample experimental evidence for olfactory preference for w males (Lenington et al., 1992). Our study, for the first time, provides experimental evidence that t females avoid t male fertilization in an actual mating context.

In w females, on the other hand, t paternity shares did not deviate from the random 50% expectations. This seems surprising, given that discriminating against t males would help w females to avoid producing sons with impaired sperm competitive ability and/or low attractiveness to females. It is, however, difficult to assess whether t avoidance is beneficial without knowing the precise underlying cost/benefit structure of the behaviour. Let b_i be the benefits of avoiding t males, where $i \in [t, w]$ denotes female genotype. Because of genetic incompatibility, we have $b_t > b_w > 0$. Let us further assume that avoiding t males is associated with a genotype-independent cost c (cost of preference or multiple mating). Even though t avoidance is beneficial for females of both genotypes ($b_i > 0$), the evolutionary relevant question is whether the overall payoff of t avoidance, i.e. $b_i - c$, is positive. Now, the asymmetry in fitness consequences for t and w females ($b_t = b_w$) may well be adaptively meaningful. Overall payoffs depend on the relative magnitude of costs c. If $c < b_w < b_t$, t male avoidance is beneficial for both female genotypes (likewise, if $c > b_t > b_w$, it is detrimental to both). There is, however, also the possibility that $b_t > c > b_w$, in which case t avoidance is adaptive for t females (as $b_t - c > 0$), but not for w females (as $b_w - c < 0$). More precise theoretical models are clearly necessary to investigate whether such a scenario can be evolutionarily stable and whether there are genetic mechanisms that could maintain such systematic preference differences between t and w females.

Pre- or postcopulatory processes? The observed fertilization bias in t females reported here can principally be the result of both pre- and/or postcopulatory processes. We did not systematically observe matings during preference tests. Instead, we used two indirect approaches to investigate whether the observed fertilization bias in t females are the result of pre- or postcopulatory mechanisms.

First, we compared observed fertilization distributions against specific, customized

null models. Despite the fact that not all multiple matings will result in multiple paternity, we expected a systematic variance difference between a scenario where all females mate with one male only (Null model 1) and a scenario where all females mate multiply (Null models 2 and 3). Observed variance estimates fell between these extreme scenarios, suggesting that both pre- and postcopulatory processes are important here. Overall, the multiple paternity rate in the experiments was 29%. This value is consistent with previous estimates on house mice (Dean et al., 2006; Firman and Simmons, 2008a; Manser et al., 2011) and confirms that female mice are actively polyandrous. It is unknown whether *t* and *w* females differ systematically in levels of polyandry. The low sample sizes did not allow for a systematic test for such a difference here. In terms of mean predictions, scenario 3 (assuming full polyandry and *t* sperm disadvantage) was most compatible with our data. This indicates, as suggested previously (Ardlie and Silver, 1996; Olds-Clarke and Peitz, 1986), that *t* males are poor sperm competitors. However, we could not quantify the fraction of males that mated with a female, but did not successfully sire offspring. The expected mean distribution of null model 3 (in Fig. 3.3B) shows that the fraction of such unsuccessful *t* males can be quite large. Another class of a postcopulatory mechanism by which females could bias fertilization towards *w* males is cryptic female choice (Eberhard, 1996). Our experimental design did not allow disentanglement of intra-male sperm competition from cryptic female choice and our results are compatible with one and/or the other. Controlled sperm competition experiments are clearly needed for reliable estimates of *t* sperm disadvantage as well as to identify the precise mechanisms that determine fertilization success.

Second, we analyzed female behaviour during the preference tests to investigate the importance of precopulatory preference. The variation in visiting preference both within (over the course of an experiment, see Supplementary Figures) and between females was substantial. Overall, we did not find any indication that *t* females avoided *t* males prior to mating. In fact, most females actively visited both males over the course of an experiment (see Supplementary Figures). The lack of clear precopulatory preferences is surprising given the series of studies repeatedly reporting *t* female olfactory preferences for *w* males (Lenington et al., 1992). It remains unclear whether females of our population are able to distinguish between *t* and *w* conspecifics. *t* haplotypes carry several MHC loci that could principally serve as indicators of male *t* status. Lindholm et al. (2013) found that *t* haplotypes were —as a consequence of recombination suppression— associated with a single, unique MHC allele. However, the role of MHC in mate choice remains controversial, both related to *t* haplotypes (Lenington, 1991)

and in general (Cheetham et al., 2007). In a very similar experiment, Rolland et al. (2003) gave females the choice between dominant and subordinate males. The resulting behaviour was analogous to the one observed here. Females actively visited and mated with both males. The authors argued that the preference for the preferred male (in this case the dominant male) was only apparent in a narrow time window during estrous. Additionally, they found that females accepted more intromissions from preferred males and mated last with the preferred males. We cannot exclude such subtle differences in female behaviour towards *t* versus *w* males here. Based on a limited sample size, we did find a weak correlation between female behavioural preference throughout the preference tests and the resulting fertilization bias. However, the fact that female visiting patterns are, to some degree, predictive of paternity outcomes, is by no means an indicator of precopulatory choice as long as there are no clear behavioural preferences. In any case, the fact that nearly uniformly distributed behavioural preferences translate into clear fertilization biases (see histograms in Fig. 3.4B) make multiple mating and sperm competition the likelier candidates to drive the observed *t* fertilization bias. The fact that null model 3 best describes the distribution of *t* paternity shares (discussed above) confirms this conjecture. Having said that, the data do not allow us to categorically rule out precopulatory choice.

The evolutionary forces that determine *t* frequencies in wild populations have puzzled biologists for more than half a century (Ardlie and Silver, 1998). It is largely unknown whether the fertilization bias observed here can be an important force for *t* frequencies in wild populations (Burt and Trivers, 2006). Female mating decisions in wild populations may not be as unconstrained as in our experiments here. If the dominant male of her territory is a *t*-carrier, a female may have to mate with him to avoid infanticide (Perrigo et al., 1991). Male dominance is an unlikely factor to explain female mating patterns here, as male territories did not overlap in our experimental setup (i.e. males could not establish a dominance hierarchy). Previous studies show that preference for dominance plays an even larger role in female mate choice decisions (Coopersmith and Lenington, 1992). Nevertheless, female mating behaviour may still be important in *t* suppression (Manser et al., 2011). Frequencies in wild populations are typically at low, but stable levels (Ardlie and Silver, 1998). In our wild study population, of which all mice used in the present experiments originated, *t* frequency has decreased significantly over a period of 5.5 years. Female mating behaviour can potentially explain this decrease (Manser et al., 2011) and paternity analyses indeed revealed a weak, but significant *t* female mate choice bias towards *t* males (Lindholm

et al., 2013). We hence have good indications that female mating behaviour may play an important role in suppressing *t* haplotypes in wild house mice. However, further analyses are necessary to specifically quantify the importance of pre- and/or postcopulatory processes in drive suppression.

In conclusion, the present study provides further evidence that genetic incompatiblities caused by SGEs, usually hidden from sight, may be an important driver of the evolution of female mating behaviour. It has been suggested that SGEs are a ubiquitous feature of life (Burt and Trivers, 2006). This study not only shows that female mating behaviour may play an important role in SGE-suppression, but also illustrates how the covert action of SGEs may help us understand important aspects of an organism's behaviour that may remain unexplained otherwise.

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Chapter 4

Sperm Competition Suppresses Gene Drive among Selection Lines in House Mice

Andri Manser, Renée C. Firman, Leigh W. Simmons, Anna K. Lindholm

Abstract

The *t* haplotype is a well-known drive element in house mice that manipulates spermatogenesis in heterozygote +/*t* males in its own favour. Based on the systematic advantage of *t* sperm within a +/*t* male's ejaculate, we expect *t* haplotypes to occur at high frequency in natural populations. Yet empirical studies measure *t* frequencies at substantially lower levels than predicted, suggesting the presence of mechanisms that suppress drive. It has been proposed that *t* haplotypes —while being strong sperm competitors within +/*t* male— compromise a +/*t* male's sperm competitive ability at the between-male level. As a result, polyandry and subsequent sperm competition may suppress drive, thereby explaining the discrepancy between theory and data. We test this polyandry hypothesis using post-hoc analysis of animals from a selection experiment. Eight selection lines were kept under monandrous or polyandrous mating conditions, respectively, over the course of 20 generations. Unknown at the time of the experiment, the *t* haplotype was present in all selection lines. In line with the polyandry hypothesis, we find that (1) +/*t* male's fertilization success is substantially reduced when in sperm competition with +/+ males and (2) *t* frequencies declined significantly in the polyandrous lines while remaining at stable, high levels in the monandrous lines. We thus demonstrate compelling evidence in support of the polyandry-suppression hypothesis.

Keywords *t* haplotype, polyandry, gene drive, meiotic drive, segregation distortion, genomic conflict, experimental evolution, sperm competition, house mouse

4.1 Introduction

We usually think of natural selection as a process favouring alleles that improve the fitness of the organisms harbouring them. Driving elements remind us that this rule can be systematically broken. These genetic entities spread through populations, often despite detrimental fitness consequences to their hosts. They achieve this by systematically biasing the fair 50:50 Mendelian inheritance ratios in their favour. This phenomenon is termed gene drive or meiotic drive (Burt and Trivers, 2006). The *t* haplotype in house mice is the textbook example of a driving element. This cluster of genes, occupying about one third of mouse chromosome 17, is detrimental to its carriers: t/t homozygotes die from recessive lethal mutations during embryogenesis (Ardlie and Silver, 1996; Lindholm et al., 2013). On its own, this would predict immediate extinction of the *t* haplotype. Yet, instead of the usual 50%, $+/t$ heterozygous males transmit the *t* haplotype to up to 90% of their progeny (Ardlie and Silver, 1996). This consistent advantage at the level of the gamete has allowed *t* haplotypes to persist in house mouse populations around the world for over 1.5 million years in spite of the harm they incur to the individuals and populations harbouring them (Hammer and Silver, 1993).

Accounting for the frequency of *t* haplotypes in natural house mouse populations is a longstanding evolutionary puzzle (Ardlie, 1998). Based on drive and homozygote lethality alone, we expect about two thirds drive carriers in the populations (Bruck, 1957). Yet empirical data from natural mouse populations suggest that *t* frequencies are considerably lower than that. This discrepancy between theoretical prediction and observation is known in the literature as the 'low *t* frequency paradox' (see Ardlie (1998) for a review). The '*t* paradox' suggests the presence of evolutionary mechanisms that suppress drive. From the viewpoint of the organism, as well as all the genes not linked to the t, this makes sense: all genes' representation in future generations becomes compromised when lethal homozygotes are produced, while only the genes on the *t* haplotype's linkage group benefit from its gametic transmission advantage. As a consequence, we predict unlinked genes to evolve mechanisms that suppress the t's selfish, harmful acts. Answering the *t* paradox may therefore not only help us understand *t* frequencies in natural populations, but also provide us with new insight into the intricacies of genomic conflict within the organism (Burt and Trivers, 2006).

More than half a century of theoretical and empirical research has brought up a multitude of solutions to the paradox, ranging from the evolution of genes that di-

rectly interfere with the mechanism at the molecular level to interdemic selection at the population level. A mechanism of drive suppression that has received a lot of attention over the past few years is polyandry, i.e. females mating with several males (see Wedell (2013) for an comprehensive review on the relationship between polyandry and gene drive). The hypothesis of polyandry as a potential suppressor of drive, originally put forward by Haig and Bergstrom (1995), is based on a simple premise. Many drive systems occur in males, and the drive mechanism often involves killing sperm that does not carry the drive chromosome. In the *t* haplotype system, $+/t$ heterozygote males produce + and *t* gametes at equal proportions. A set of distorter loci interferes with the flagellar function of all sperm (+ and *t*), while *t* sperm swimming ability is locally restored by a responder (akin to a poison-antidote system, see Herrmann and Bauer (2012)). Such targeted killing makes drive-carrying sperm, by definition, successful against rival sperm within a male's ejaculate. Yet, it typically results in drive males producing few viable sperm (Price and Wedell, 2008). As a result, the few sperm from drive bearing males are often outcompeted by the more numerous sperm from non-driving males in sperm competition between ejaculates. Controlled sperm competition experiments in a number of taxa have confirmed this conjecture: driving elements are often detrimental to a male's sperm competitiveness —including impaired success of those sperm that carry the drive element (Price and Wedell, 2008).

The link between drive and male sperm competitive ability bears two interesting implications. First, we predict that polyandry will suppress drive frequency in populations where female multiple mating is common. In the context of the t haplotype, this may help us resolve the 'low *t* frequency paradox'. Second, if a female can 'invite' sperm competition to reduce the proportion of offspring inheriting a harmful drive gene, we can expect coevolution between drive and the tendency of females to mate multiply (see Holman et al. (2015) for a recent model). Gene drive has therefore been proposed as one of many explanations for the evolution of polyandry (Wedell, 2013).

In the present study, we were able to test key predictions of the polyandry hypothesis due to a unique set of circumstances. A post-hoc analysis of individuals from a selection experiment on wild-caught Australian house mice (*Mus musculus domesticus*) revealed that the *t* haplotype was present at considerable frequencies in all selection lines, but this was unknown at the time. In the experiment, mice were kept under an either strictly monandrous or polyandrous mating regime in 8 selection lines over the course of 20 generations (Firman and Simmons, 2008c). This allowed us to ask two questions concerning polyandry and drive suppression. In a first step, we ask how the

t haplotype affected sperm competitiveness within and between ejaculates, using data from a sperm competition experiment conducted on animals that originated from the selection lines (Firman and Simmons, 2011). In a second step, we explore how the mating regime (monandry vs. polyandry) affected *t* frequency dynamics in the selection lines. Observed frequency dynamics are then compared to the predictions of a model based on parameter values estimated in step one.

4.2 Material and Methods

The Study System

Selection Experiment Over the course of 20 generations, a total of 8 replicate lines of mice were subjected to either a monandrous or a polyandrous mating regime (see (Firman and Simmons, 2008b) for a detailed description of the selection experiment). Four replicate lines were mated monandrously (*M*-lines) via a middle-class neighbourhood design. Each *M*-line consisted of 18 males and 18 females and each fecund pair contributed one randomly selected male and female to the next generation. Hence, all individuals of *M*-lines had the same fitness, eliminating both natural and sexual selection. Four replicate lines were mated polyandrously (*P*-lines). Each *P*-line again consisted of 18 females and 18 (potentially >18) males. Here, each female was subsequently mated to three different males, where a set of three males each mated with a set of three females (see Fig. 1 in Firman and Simmons (2009) for an illustration of the mating design). As in the *M*-lines, one male and one female offspring were randomly selected to advance to the subsequent generation with the aim of removing natural and precopulatory sexual selection (both *M*- and *P*-lines equalized mating success across females). The key difference between *M*- and *P*-lines was that the polyandrous treatment allowed for postcopulatory sexual selection: males competed over fertilization, and the number of males who contributed to successive generations was determined by the relative fertilization success of a given male.

The t haplotype in the selection lines For this study, we reanalyzed tissue samples from animals originating from selection experiment for the presence of the *t* haplotype. Altogether 1092 individuals, originating from generations 0, 12, 16, and 19 were typed at the *Hba-ps4* locus. This marker is diagnostic for the *t* haplotype as it contains a 16 basepair *t* haplotype specific insertion (Schimenti and Hammer,

1990). We found that the t haplotype occurred at considerable frequencies in all eight selection lines. None of the 1,092 samples was t/t homozygous, strongly suggesting that the t haplotype present in the selection lines carried a recessive lethal mutation common to all copies of the t haplotype.

Measuring Sperm Competition Parameters

The two parameters of interest We developed a model to determine the extent to which the two different mating regimes (i.e. one versus three male mating partners) affected the frequency of the t haplotype among the selection lines. The model, which is provided in detail in supplementary text S1, depends on two crucial parameters of sperm competitiveness (see Haig and Bergstrom (1995)): (i) Parameter d defines drive, i.e. the probability that a genetic offspring of a $+/t$ male inherits the t allele. If $d = 0.5$, sperm transmission ratio is perfectly Mendelian (Null hypothesis). If $d = 1$, the t haplotype is transmitted to all offspring. Alternatively, d can be thought of as the competitiveness of t sperm within a $+/t$ male. (ii) Parameter c measures the relative competitiveness of $+/t$ males, defined as , compared to $+/+$ males (whose competitiveness equals unity). c can thus be interpreted as the competitiveness cost of a $+/t$ male's sperm in competition between males. If $c = 0$, $+/+$ and $+/t$ males do not differ in their sperm competitive ability (Null hypothesis 1). However, $+/t$ may have a reduced sperm competitiveness as a direct consequence of drive, because the t haplotype kills a considerable fraction of a $+/t$ male's sperm. Assuming a fair raffle model where a male's competitiveness is proportional to his number of viable sperm, competitiveness cost c can be expressed directly as a function of drive parameter d, with $c[d] = \frac{d-0.5}{d}$ (Null hypothesis 2). For example, if drive is complete ($d = 1$), half of a $+/t$ male's sperm (i.e. all + sperm) will be killed, and as a result his sperm competitiveness is expected to be halved (since $1 - c[1] = 0.5$).

Sperm competition experiment To estimate the two relevant sperm competition parameters d and c, we used data from a sperm competition experiment conducted on sexually mature mice of generation 12 of the selection lines (see Firman and Simmons (2011) for a detailed description of the experimental method). In this experiment, two males —one M- and one P-male from the four M- and P-lines— were mated to a single M- or P-females using a semi-factorial design, creating a total of 32 ordered male combinations (16 $M \times P$ and 16 $P \times M$). Specific male combinations were randomly assigned to females of either selection history, excluding matings between

males and females from the same replicate lines (to avoid confounding effects of co-evolution within lines). The entire design was replicated, resulting in 64 experimental matings. Females in oestrus were mated to a given male and checked half-hourly for the presence of a mating plug. The plug was removed upon detection and females were then paired with the second male. Females were again checked in half-hour intervals for a mating plug and removed after a successful second mating. 14 days after mating, females were sacrificed and embryos were removed from the reproductive tract. Paternity analyses were conducted previously (Firman and Simmons, 2011) and allowed us to determine the fertilization success of either male. In the present study, we additionally determined the t genotype of all potential parents and offspring (using the method described above). Of the 192 potential parents and 495 embryos from the experimental mating crosses, the t genotype was successfully assigned to 190 parents and 493 embryos. None of the 493 embryos were t/t homozygous, suggesting that genotypic outcomes of each mating cross were measured after t/t lethal embryos were resorbed.

Estimation procedure We used a maximum likelihood approach (using the `mle2` function in the `bbmle` package in R, R Core Team (2014)) to find the best estimates for parameters d and c given the paternity and t frequency outcomes of the experimental mate crosses. Note that —depending on the genotypes involved in a particular cross— we expect different mating outcomes for a given set of parameter values d and c. Writing our own, customized likelihood functions allowed us to derive single parameter estimates using data across different mating combinations (see Supplementary Table S4.1 for an overview).

Estimating drive, d Ideally, drive is estimated in the absence of sperm competition between males, that is in monogamous mating crosses that involve $+/t$ males only. In this study, experimental mating crosses always involved two males that competed over fertilization. However, under the assumption that the genotypic outcome within the subset of offspring sired by a given male is independent of sperm competitive effects between ejaculates, we can look at each subset sired by a given male as if mated monogamously. Accordingly, we used P_{within}, defined as the proportion of $+/t$ heterozygotes (based on t genotype information) among all viable offspring sired by a given $+/t$ male (based on paternity information), as a our response variable to estimate drive parameter d.

Note that, for a given level of d, P_{within} will depend on the female genotype (see Equation S4.3 for a general derivation where $P_{within} = y_{m,1,1}$). In a cross between a $+/+$ female and a $+/t$ male, male drive alone determines the proportion of $+/t$ offspring

and we have $P_{within} = d$. In a cross between $+/t$ female and a $+/t$ male, both males and females may provide t gametes and a fraction will die during embryogenesis due to t/t lethal effects, resulting in $P_{within} = \frac{1}{2-d}$. These two expressions were used to search for the parameter value d that best fitted the observed P_{within} values using a maximum likelihood estimation (using the `mle2` function in the `bbmle` package in R, R Core Team (2013)). The proportion of $+/t$ offspring was assumed to follow a beta-binomial distribution, which allowed us to account for potential overdispersion with parameter θ. In a full model, we allowed drive estimates to vary as a function of female genotype ($+/t$ or $+/+$), the order of the male ($1^s t$ or $2^n d$), male selection history (M or P) and their interaction. Competing models of decreasing complexity where then compared based on AIC values.

Estimating sperm competitiveness cost, c To estimate between ejaculate parameter c, we analysed the proportion of viable offspring sired by a $+/t$ male when competing against a $+/+$ male, denoted by $P_{between}$, as a response variable. Due to embryo mortality and different t frequency among female gametes, we again expect different values $P_{between}$ depending on female genotype (see Equation S4.4 where $P_{between} = P^t_{m,2,1}$). We used this equation to find the best estimate of parameter $\hat{c}$ given the observed data, again using maximum likelihood. $P_{between}$ was again assumed to follow a beta-binomial distribution. We started parameter estimation with a full model, where c could vary as a function $+/t$ male order (1^{st} or 2^{nd}), male selection history (M and P), their interaction, and female genotype ($+/+$ or $+/t$). Again, we performed model selection in a backward fashion using AIC values.

Litter sizes Due to t/t embryo mortality, we expected $+/t$ females to suffer from a reduced litter size in any mating cross with successful $+/t$ fertilization. We analysed litter sizes as function of the proportion of the offspring sired by a $+/t$ male and female genotype in a generalized linear model (GLM) using an exponential link function and a Poisson error distribution. Note that such litter size losses, even if present, will not affect t frequency dynamics in the selection lines since every female contributed a standardized number of two offspring to the subsequent generation.

t Frequency Dynamics in the Selection Lines

General deterministic model predictions The general theoretical argument as to how sperm competition affects t frequencies has been presented previously Haig and Bergstrom (1995). Here, we have tailored these previous models to the specific

circumstances in the selection lines. In Supplementary Text S1, we derive how t frequency in the selection lines, denoted by Δy, will change as a function of the number of partners a female mates with (n) and the two sperm competition parameters d and c in an population of infinite size (deterministic model, equation S4.5).

Observed t frequency dynamics To test whether the mating regime (monandry vs. polyandry) affected t frequency dynamics in the selection lines, we analyzed the observed frequency of $+/t$ genotypes $\hat{y}$ as a function of treatment (M or P), time (generations $g \in 0, 12, 16, 19$), and their interaction in a generalized linear mixed effects model (GLMM) using a logit link function and a binomial error distribution. We fitted random intercept and slope for each selection line to allow for variation among the eight independent lines, both in terms of starting frequency (random intercept) and frequency change (random slope).

Predicted t frequency dynamics We used a stochastic version of the model described in Supplementary Text 4.5 to predict t frequency dynamics in the selection lines consisting of finite populations of 36 individuals (sampling from binomial distributions). The stochastic model was parameterised using the best estimates $\hat{d}$ and $\hat{c}$ (see Table S4.1), and we used $n = 1$ and $n = 3$ for the monandrous and polyandrous selection lines, respectively. Starting with average observed $+/t$ genotype frequencies at generation 0, we then calculated 10^5 evolutionary trajectories for all eight selection lines.

Comparing observed and predicted dynamics The fit of the parameterized model predictions to the observed frequency dynamics was assessed in two ways. Firstly, we calculated the likelihood of our fully parameterised, deterministic model predictions given the observed data (using the `mle2` function). Secondly, we calculated new maximum likelihood estimates for parameters d and c as suggested by the observed t frequency dynamics in the selection lines. A comparison between the parameter estimates based on the competitive mating crosses (see above) and the parameter estimates based on the frequency dynamics can be seen as a different way to quantify the accuracy of our model.

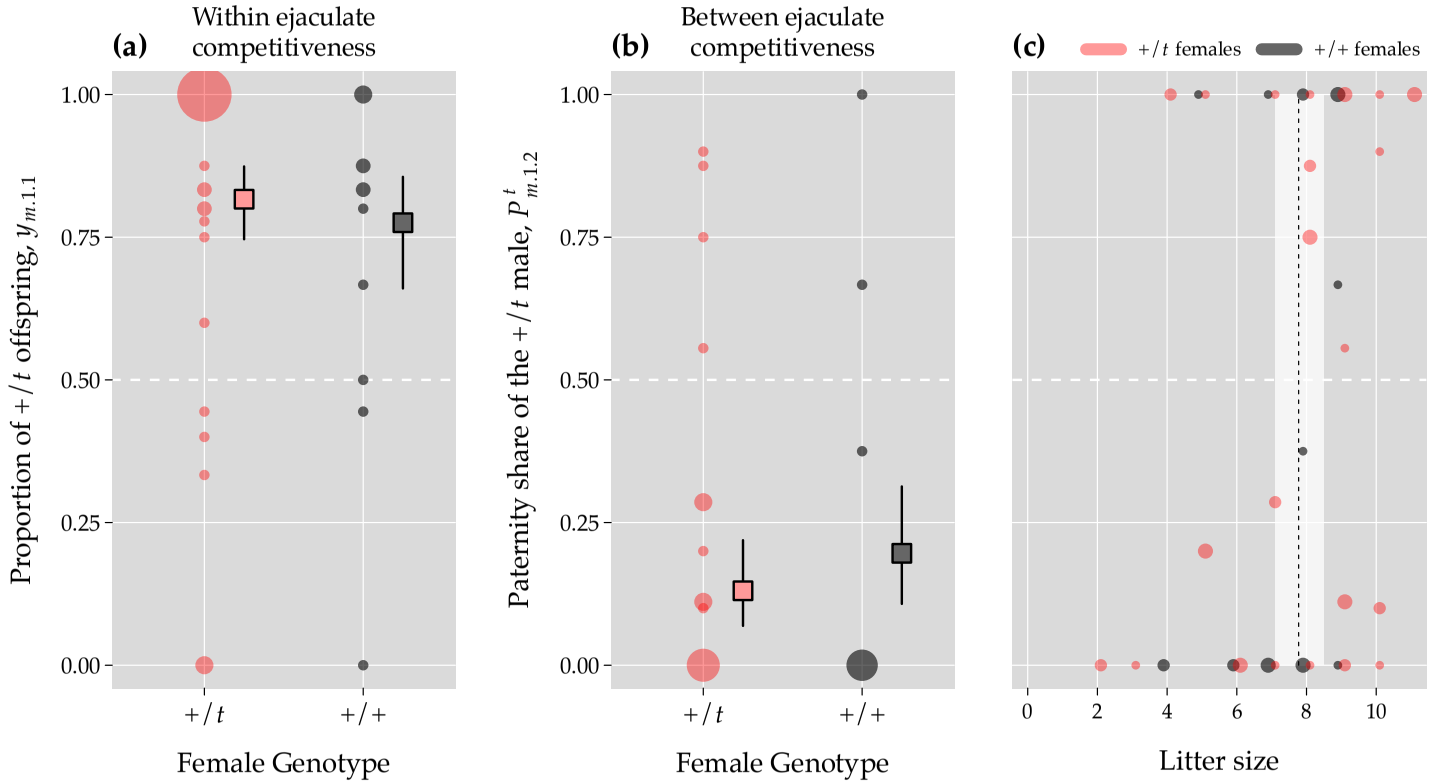

Figure 4.1. (a) Proportion of $+/t$ heterozygotes among all viable offspring sired by a given $+/t$ male as a function of female genotype. Squares and arrows show model predictions including 95% CI based on the maximum likelihood estimation of parameter d. (b) Paternity share of a $+/t$ male when competing against $+/+$ male as a function of female genotype. Squares and arrows show model predictions including 95% CI based on the maximum likelihood estimation of parameter c. (c) Litter sizes as a function of the proportion of the litter that was sired by a $+/t$ male. The dotted line and the white shaded area illustrate GLM predictions including the 95% CI. In all panels, the surface area of the dots represents the number of observations for a given x, y combination. Black color is used to depict mating crosses that involve $+/+$ females, red colors are used for mating crosses with $+/t$ females.

4.3 Results

Parameter Estimation

Levels of drive, d In 53 cases, a $+/t$ male successfully sired at least one of the analyzed embryos, allowing us to calculate proportion of $+/t$ offspring among his progeny P_{within} (see Fig. 4.1a). According to the model selection procedure, drive levels d did not systematically vary as function of mating order, male selection history, or female genotype. In the minimal adequate model, we were thus left with a context-independent drive estimate of $\hat{d} = 0.78$ (95% CI: $[0.66, 0.86]$). As suggested by the confidence interval, this estimate significantly deviated from Mendelian expectations ($z = 4.43$, $P < 0.001$ against $H_0 : d = 0.5$).

Sperm competitiveness cost, c In 34 experimental mating crosses, a $+/t$ male competed over fertilization with a $+/+$ male, allowing us to calculate the $+/t$ male's fertilization success (see Fig. 4.1b). Based on model selection, neither male selection history nor female genotype had an influence on sperm competitiveness c. Mating order, on the other hand, played an important role with the first male achieving a significantly larger share of a litter's paternity ($z = 2.98$, $P < 0.01$). More importantly, $+/t$ sperm competitiveness was substantially reduced if compared to $+/+$ males: corrected for the mating order effect, the maximum likelihood method yielded a parameter estimate of $\hat{c} = 0.76$ (95% CI: $[0.54, 0.88]$). This estimate was significantly different from Null hypothesis 1 ($z = 4.24$, $P < 0.001$ against $H_0 : c = 0$) and marginally non-significant from Null hypothesis 2 ($P < 0.01$ against $H_0 : c = \frac{\hat{d}-0.5}{\hat{d}} = 0.56$).

Litter sizes Fig. 4.1c illustrates litter sizes as a function of the proportion of the litter that was sired by a $+/t$ male. Surprisingly, the proportion of $+/t$ fertilization did not affect litter sizes of $+/t$ females: both explanatory variables (female genotype, proportion $+/t$ male sired) were removed during model selection, leaving us with a simple intercept model with an average litter size of 7.77 (95% CI: $[7.10, 8.49]$).

t Frequency Dynamics in the Selection Lines

General model predictions The model description in the supplementary material summarizes how t frequencies in an infinitely large population depend on drive levels d, sperm competitive cost c and the number of female mating partners n. In general, drive creates selection for t haplotypes at the gamete level, while t/t lethality

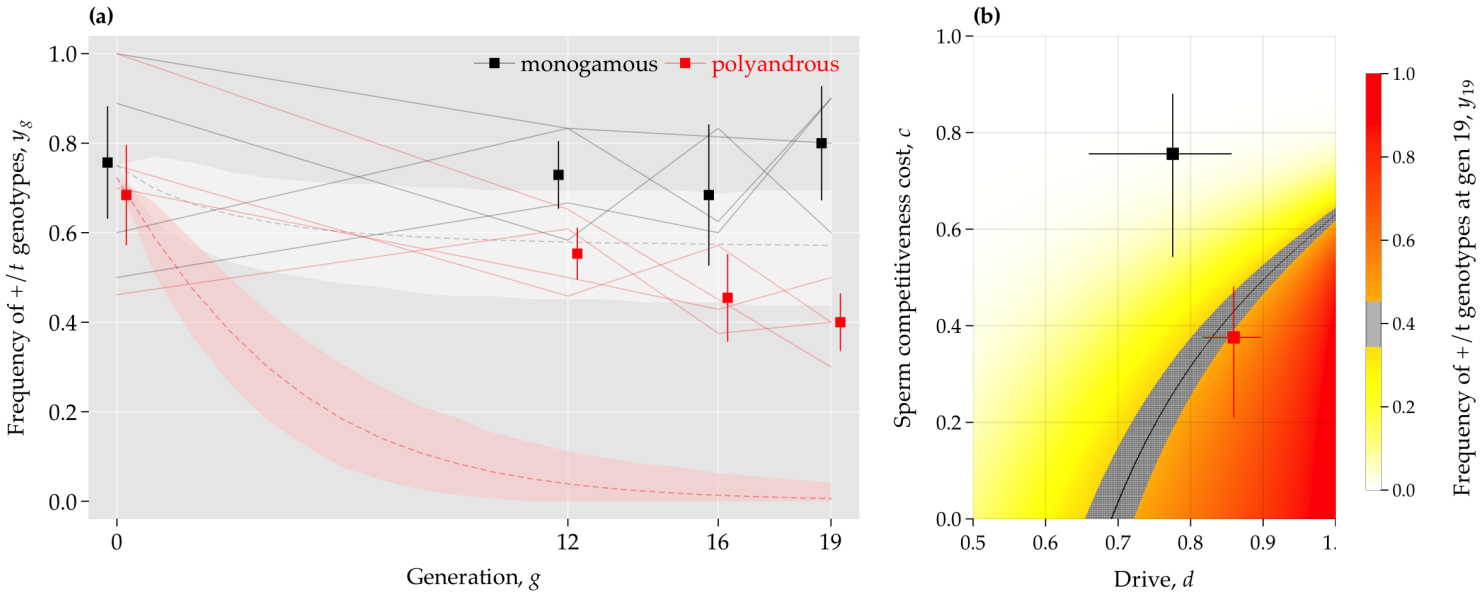

Figure 4.2. (a) Observed and predicted $+/t$ genotype dynamics for the monandrous (black/grey) and polyandrous (red) selection lines. Grey lines show observed genotype frequency dynamics in the four monandrous selection lines. Red lines show frequency dynamics in the four polyandrous lines. Squares and arrows depict mean and standard error of the observed frequencies in a given generation over all four selection lines of a given selection treatment. Dotted lines and shaded areas show stochastic model predictions based on parameter estimates and from the sperm competition experiment. (b) Deterministic model predictions for genotype frequencies at generation 19 in the polyandrous lines as a function of sperm competition parameters c and d. The grey area corresponds to the observed frequency after 19 generations. The black square shows parameter estimates and including 95% CI (based on the sperm competition experiment). The red square shows parameter estimates and based on the observed frequency dynamics. Supplementary Table Table S1. Structure of the models used to derive estimates for parameters d and c using a Maximum Likelihood. Letter is used to denote the i-th observation, whereas m defines the female genotype of a given cross (with m=0 for +/+ females and m=1 for $+/t$ females).

and between male sperm competition result in selection against the t. Frequencies will eventually end up at a stable, steady-state equilibrium where the three selective forces are in balance. As expected, higher drive levels d will increase equilibrium frequency, whereas a larger sperm competition cost c will lower it. The more males a female mates with (n), the more equal the contribution of each male, the greater the importance of between ejaculate competition. As a result, equilibrium t frequency will decrease with larger n. Note that t frequencies are confined to the range between 0 and 0.5 (the point where all individuals are $+/t$ heterozygous) due to recessive t/t lethality.

Observed t frequency dynamics Figure 4.2a shows the observed and predicted frequency dynamics of $+/t$ individuals as a function of the mating regime (monandry vs. polyandry). According to the GLMM, $+/t$ genotype frequency significantly decreased in the polyandry lines ($z = \breve{}2.53$, $P < 0.05$), while remaining constant in the monandry lines ($z = 0.15$, $P = 0.88$).

Comparing observed and predicted dynamics These observations are qualitatively in line with the stochastic model predictions (see dotted lines and shaded areas in Fig. 4.2a) which also predict lower t frequencies in the polyandrous lines. However, observed t frequencies in the polyandrous lines did not decrease nearly as dramatically as predicted by the model. According to the modelled dynamics, the t haplotype frequencies in the P-lines should have gone to extinction in most simulation runs. Figure 4.2b summarizes this result from a different angle. It shows deterministic model frequency predictions at the end of the selection experiment (generation 19) as a function of sperm competition parameters c and d. Again, parameter estimates from the sperm competition experiment do not overlap with both the observed frequencies at generation 19, nor with the parameter estimates c and d based on the observed frequency dynamics.

4.4 Discussion

Our study validates two key predictions regarding polyandry and sperm competition as a suppressor of gene drive in the t haplotype system of house mice. Firstly, we show that $+/t$ males were heavily compromised in their sperm competitive ability when competing against $+/+$ males. Secondly, we show that this systematic $+/t$ male disadvantage in sperm competition significantly affected t haplotype frequencies dynamics in a

selection experiment conducted over 20 successive generations. t frequencies declined significantly in those selection lines that were maintained under a strict polyandrous mating regime, and yet constantly remained at high levels among selection lines in which mice were bred monogamously. The observed t frequency dynamics are qualitatively in line with our model predictions. However, the observed t frequency decline in the polyandrous lines was not as severe as expected based on our parameter estimates.

Reduced sperm competitiveness We have found that $+/t$ males are severely impaired in their sperm competitive ability. Our best estimate of $\hat{c} = 0.76$ suggests that $+/t$ males only managed to fertilize, on average, 19% of a litter when competing against a $+/+$ male (before embryo mortality). The effect of the t haplotype on a male's sperm competitiveness has only rarely been measured previously. The most robust estimate of $+/t$ male sperm competitiveness based on a large sample size was provided very recently by Sutter and Lindholm (2015), who reported a $+/t$ male paternity share as low as 11%. Most previous estimates were based on extremely limited sample sizes. Nevertheless, they all report similar $+/t$ male sperm disadvantage, with paternity share ranging between 17% and 22% (Olds-Clarke and Peitz, 1986; Manser et al., 2011; Ardlie and Silver, 1996). Strikingly, all estimates suggest that $+/t$ males are worse sperm competitors than one would expect based on the killing of wildtype sperm alone (Null hypothesis 2). This implies that the poison-antidote system is not perfectly fine-tuned. As a result, not only $+$ sperm, but also t sperm appear to be affected in their swimming ability.

The systematic disadvantage of drive carrying males in sperm competition against other males is not an isolated observation, but a recurring pattern across drive systems (Price and Wedell, 2008). For example, similar reductions in drive male's paternity shares have been reported sex chromosome drive systems of invertebrates (Atlan et al., 2004; Price et al., 2008a; Wilkinson and Fry, 2001) and plants (Taylor et al., 1999). This is a likely a consequence of similarities in the drive mechanisms, which often use sperm as the target of their attack.

Fitness consequences We found that $+/t$ females did not suffer any litter losses when fertilized by $+/t$ males. This is surprising, given the fact that t/t embryos die during embryogenesis (Lindholm et al., 2013). A power analysis showed that sample sizes would have been sufficient to detect expected litter losses given observed drive levels . We can only speculate why this was not the case here. One possibility is that mice used in the selection experiment carry a t haplotype variant with a lethal mutation

that acts early during embryonal development, i.e. before the embryos are implanted into the uterus (which occurs at day 4 of pregnancy, ref.). If more embryos are produced than can be implanted, females may only select embryos that are developing normally, thereby avoiding t/t related litter losses. At least 16 different t haplotypes have so far been identified (Klein et al., 1984), each containing lethal mutations that act at different stages of embryonal development. One complementation group (t_{12}) has been shown to act before day 4 of pregnancy (Bennett, 1975). Interestingly, early studies suggest that this t haplotype variant is associated with drive levels around 75% (Smith, 1956; McGrath and Hillman, 1980), a value that is strikingly close to our estimates here. However, the same studies also report reduced litter sizes in crosses between $+/t_{12}$ heterozygotes. Note that the absence of female litter reduction, irrespective of its causes, did not affect our t frequency predictions in the selection lines, because the litter size of all females was standardized to two.

Explaining the t paradox: polyandry as a suppressor of drive frequency

One important consequence of the systematic disadvantage of drive-carrying males is the effect of polyandry on drive frequency dynamics. Understanding the factors that explain drive frequency dynamics in natural populations is a longstanding focus in drive research, both in the t haplotype system as well as in drive systems in general (Ardlie, 1998). This is the first study that directly examined the impact of female mating partner number on the t dynamics in a controlled setting. We show that polyandry resulted in a significant decrease in t frequency in four independent selection lines, thereby providing the first direct empirical evidence for polyandry as a drive frequency suppressor in this system.

In natural populations, t haplotypes are typically found at markedly lower frequency than predicted by theory (referred to as the t frequency paradox). Empirical evidence from both laboratory and natural populations suggest that house mice are markedly polyandrous (Dean et al., 2006; Firman and Simmons, 2008a; Manser et al., 2011), with multiple paternity rates ranging between 20 and 30%. The results of this study, together with the polyandry rates in natural populations, suggest that polyandry may be an important factor in explaining the t frequency paradox. Moreover, polyandry mediated drive suppression may explain an additional pattern found in natural populations. t frequencies are typically negatively correlated with population size (Ardlie and Silver, 1998), precisely the pattern one would expect if polyandry rates increase with increasing population size (as the number of mating opportunities increase, see Dean et al. (2006)).

Interestingly, the drop in t frequency was not as marked as predicted by our parameterised model. Principally, we see two explanations for this discrepancy between theory and data. Firstly, our model predictions could be based on faulty parameter values, that is systematic measuring errors during parameter estimation. Fig. 4.2b suggests that, even if the uncertainty in our parameter estimates is taken into account (see 95% CI arrows), the predicted frequencies would not match the observed frequencies by the end of the selection experiment. The model predictions are based on parameter estimates d and c using the paternity outcomes of a single-generation sperm competition experiment (see black square and arrows in Fig. 4.2b). Alternatively, the entire selection experiment can be regarded as a whole series of sperm competition experiments, repeated over 20 generations. According to this logic, one may consider the parameter estimates which are based on the t frequency dynamics of the entire selection experiment (see red dot and arrow in Fig. 4.2b) as more reliable and robust estimates for d and c.

Secondly, the discrepancy between the model and data could also be the result of biologically meaningful deviations from the modelling assumptions. Several simplifying assumptions would probably only affect the variance predictions of the model. For instance, the observed male mating order effects (see above) are likely to affect variance predictions. Other simplifying model assumption may result in faulty mean predictions. One might, for example, expect that both parameters c and d are not constant (as assumed in the model), but evolve over the course of the selection experiment. Males with lower sperm competitiveness costs c and/or lower drive levels d would certainly be more successful in an polyandrous selection lines, where postcopulatory selection plays an important role. As a result, values of c and d may decrease over the course of the experiment. However, we estimated parameters c and d from individuals from generation 12, a point where selection on c and d already should have occurred. Hence, if anything, parameter values were even larger at the beginning of the experiment. Moreover, we did not find that drive levels d were dependent on male selection history (note that parameter c was not estimated as a function of selection history, because P-males were always competing against M-males). Hence, we have no convincing explanation for the discrepancy between theoretical prediction and observed frequency dynamics, and the question certainly deserves further investigation.

Does gene drive promote the evolution of polyandry? Female multiple mating may not only help us understand drive gene dynamics in natural populations, as shown here, but conversely drive genes may also have implications for the evolution

of polyandry itself. While mating with several males is usually associated with considerable fitness cost to females (e.g. Chapman et al. (1995), benefits of female multiple mating are less obvious (as one male is usually sufficient to ensure reproductive success). A theoretical study has recently investigated the co-evolutionary dynamics of polyandry and sex chromosome drive Holman et al. (2015). It shows that polyandry can result in the evolution of polyandry, but only if the drive gene is associated with homozygote fitness costs. The experimental setup here did not allow us to test the effect of drive on polyandry rate, as the number of mating partners was held constant. Moreover, while homozygote costs certainly exist in the t haplotype system, our data here suggest that $+/t$ females did not suffer from fitness losses, even if the entire litter was sired by a $+/t$ male (see above). Hence, while drive avoidance may certainly be a factor that can promote polyandry, it appears unlikely in the specific case studied here.

Finally, this study is a striking example for how the hidden action of a drive system can, unknowingly, have large-scale effects in an experiment. Previous work published on the selection experiment reported an increased sperm competitive ability and sperm quality of males from the polyandrous selection lines (Firman and Simmons, 2009, 2011). Here, we show that this effect can partly be attributed to the t haplotype, thus providing a mechanistic explanation for the previously published effects. However, note that P-males outcompeted M-males in competitive mate crosses between males of the same t genotype (i.e. between two $+/+$ or two $+/t$ males, see Supplementary Figure S1). This suggests selection on additional traits related to sperm competition that are independent of the t. Due to the absence of obvious phenotypic effects, drive systems are often inherently difficult to detect, requiring in depth cytological or genomic work over multiple generations (Burt and Trivers, 2006). The identification of gene drive was possible here, because the experiments were conducted on one of the best-studied, genetic model organisms. Thanks to the genomic revolution, new drive systems across a broad range of taxa are being described at an ever-faster rate. These discoveries suggest that drive is not a rare, isolated phenomenon but widespread across diploid life (Burt and Trivers, 2006). Our study demonstrates that uncovering the hidden action of these genetic outlaws is a worthwhile endeavour. In the worst case, they may help us identify hidden, unintended side effects in our study systems. In the best case (as was the case here), they may provide us with a deeper, more mechanistic understanding of the evolutionary processes under examination.

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4.5 Supplementary Material

Deterministic Model

The basic theoretical argument as to how polyandry affects the frequency of t haplotype frequency in a population has been developed elsewhere. Haig and Bergstrom (1995) considered the two most extreme cases, where a female either mates with one male only (monogamy) or with the entire male population. In Manser et al. (2011), females were assumed to mate with one or two males. Here, we extend the previous frameworks to tailor them to the specific situation in the selection experiment. First, we consider a case where females mate with an arbitrary number of mating partners n. Second, we assume that all females have an equal litter size at birth (as two of her offspring where used in the next generation irrespective of her mating partners). This is in contrast to previous models where $+/t$ may suffer from a reduced litter size due to t/t embryo mortality.

To model how the number of female mating partners n affects t frequency dynamics, let y be the frequency of $+/t$ heterozygote adult individuals in the selection experiment in the current generation g. Note that, since t/t are not viable, the frequency of $+/+$ homozygotes is simply given by $1-y$. To calculate the frequency change of $+/t$ individuals Δy from the current (g) to the next, non-overlapping generation ($g+1$), individuals in the selection lines undergo the following life cycle.

Mating First, individuals of the present generation are mated in a random fashion. Let $f_{m,n,k}$ denote the mating frequency between a female of genotype m (with $m=0$ if she is $+/+$ and $m=1$ if she is $+/t$) and n males of which k are $+/t$ heterozygotes. The probability, that a female encounters k $+/t$ males in her sample of n mating partners follows a binomial distribution. For a given mating combination m,n,k, we thus have

$$f_{m,n,k} = y^m(1-y)^{1-m}\binom{n}{k}y^k(1-y)^{n-k}. \tag{S4.1}$$

For example, the frequency of a t female ($m=1$) encounters two $+/t$ males ($k=2$) out of three males ($n=3$) will be $f_{1,3,2} = 3y^3(1-y)$.

Gamete production and sperm competition The genotypic outcome of a given mating combination m,n,k will depend on the probability that a given female egg cell carries the t, denoted by e_m, as well as the probability of fertilization by a t sperm, denoted by $s_{n,k}$. In females, segregation ratios are Mendelian, we hence have $e_m = \frac{m}{2}$.

Among the n males, $+/t$ and $+/+$ males contribute the fraction $\frac{k}{n}$ and $\frac{n-k}{n}$, respectively, to the sperm puddle (viable and non-viable). However, only $(1-c)$ of a $+/t$ male's sperm is viable and only a fraction d carries the t. $s_{n,k}$ is thus given as the fraction of viable t sperm $\frac{k}{n}d(1-c)$ over the total amount of viable sperm $\frac{n-k}{n}+\frac{k}{n}(1-c)=1-\frac{k}{n}c$. If all viable sperm have an equal fertilization probability, we have

$$s_{n,k}=\frac{\frac{k}{n}d(1-c)}{1-\frac{k}{n}c}. \tag{S4.2}$$

If there are only $+/t$ males in a given male sample, $s_{n,k=n}=d$. Likewise, if all males are $+/+$, $s_{n,k=0}=0$. Hence, a $+/t$ male's reduced sperm competitiveness (c) is only relevant if males of both genotypes are in the sample ($0<k<n$ if $n>1$).

Offspring production Based on e_m and $s_{n,k}$, we can now derive the proportion of $+/t$ offspring in a given mating cross at birth $y_{m,n,k}$ (after t/t embryo mortality). Heterozygote $+/t$ zygotes form if a t egg is fertilized by a $+$ sperm (at frequency $e_m(1-s_{n,k})$) or if a $+$ egg is fertilized by a t sperm (at frequency $(1-e_m)s_{n,k}$). To obtain $y_{m,n,k}$, we simply divide the frequency of $+/t$ heterozygotes by the total amount of live offspring at birth. Because a fraction $e_m s_{n,k}$ perish *in utero*, the total number of offspring at birth is $1-e_m s_{n,k}$. We thus have

$$y_{m,n,k}=\frac{e_m(1-s_{n,k})+(1-e_m)s_{n,k}}{1-e_m s_{n,k}}. \tag{S4.3}$$

It is also useful to calculate $P^t_{m,n,k}$, defined as the proportion of live offspring of a $+/t$ male when mating with a female of genotype m and competing against $n-1$ other males of which $k-1$ are also $+/t$ heterozygous (for $k\geq 1$). A single $+/t$ male will fertilize $\frac{1}{n}(1-c)$ egg cells, of which $1-e_m s_{1,1}$ survive the embryonic stage. We have

$$P^t_{m,n,k}=\frac{\frac{1}{n}(1-c)(1-e_m s_{1,1})}{(1-e_m s_{n,k})(1-\frac{k}{n}c)}. \tag{S4.4}$$

Frequency dynamics To derive the change in $+/t$ frequency $\Delta y(n,c,d)$ between generation g and $g+1$ if females mate with n partners, we simply multiply the frequency of a given mating (equation S4.1) with the proportion of $+/t$ it produces (equation S4.3). If summed over all possible mating combinations, we have

$$\Delta y = \sum_{m=0}^{1} \sum_{k=0}^{n} f_{m,n,k} y_{m,n,k} - y. \tag{S4.5}$$

	Drive, d	**Sperm competitiveness cost, c**
Observed outcome	$P_{obs,i}^{within} = \frac{n_i^{+/t\|+/t}}{n_i^{+/t}}$	$P_{obs,i}^{within} = \frac{n_i^{+/t\|+/t}}{n_i^{+/t}}$
Expected outcome	$P_{exp,i}^{within} = (1-m_i)d_{m=0} + m_i \frac{1}{2-d_{m=1}}$	$P_{exp,i}^{between} = (1-m_i)\frac{1-c_{m=0}}{2-c_{m=0}} + m_i \frac{(1-c_{m=1})(2-d)}{4-2c_{m=1}-d-dc_{m=1}}$
Linear Predictors (Full Model)	$d_{m=0} = \alpha_1 + \beta_1 *$ mating order $+$ $\beta_2 *$ selection history $+ \beta_3 *$ history * order $d_{m=0} =$ $\alpha_1 + \alpha_2 + \beta_1 *$ mating order $+ \beta_2 *$ selection history $+ \beta_3 *$ history * order	$c_{m=0} = \alpha_1 + \beta_1 *$ mating order $+$ $\beta_2 *$ selection history $+ \beta_3 *$ history * order $c_{m=0} =$ $\alpha_1 + \alpha_2 + \beta_1 *$ mating order $+ \beta_2 *$ selection history $+ \beta_3 *$ history * order
Link function	logit(d)	exp(c)+1
Distribution	$P_{obs,i}^{within}\ Betabinom(Prob = P_{exp,i}^{within}, \theta)$	$P_{obs,i}^{between}\ Betabinom(Prob = P_{exp,i}^{between}, \theta)$
Sample size	53	34
Remaining predictors after MS	$\alpha_1 = 1.23$, $z = 4.43$, $P < 0.001$ $\theta = 3.45$, $z = 1.96$, $P < 0.05$	$\alpha_1 = 1.41$, $z = -4.24$, $P < 0.001$ $\beta_1 = 1.95$, $z = -2.99$, $P < 0.01$ $\theta = 1.60$, $z = 2.37$, $P < 0.05$
Estimates and 95% CI	0.78 [0.66, 0.86]	0.76 [0.54, 0.88]

Table S4.1. Structure of the models to derive estimates for parameters d and c using Maximum Likelihood. Letter i is used to denote the i-th observation, whereas m defines the female genotype of a given cross (with $m = 0$ for $+/+$ females and $m = 1$ for $+/t$ females).

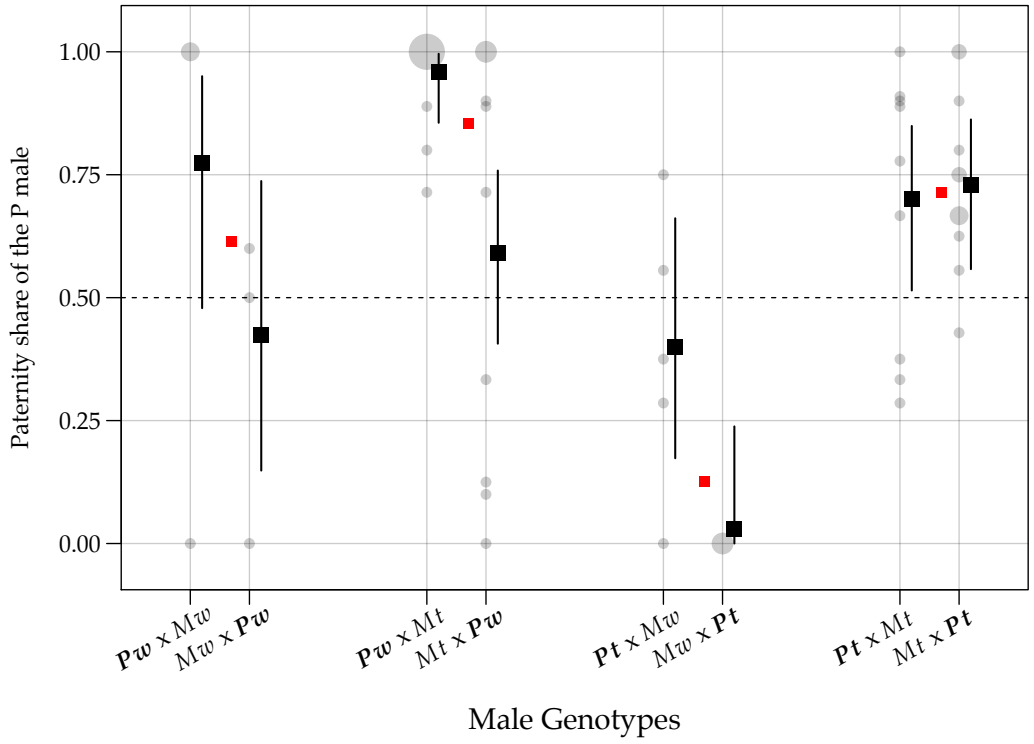

Figure S4.3. Paternity share of a *P* male when competing against *M* male as a function of male genotype. Squares and arrows show model predictions including 95% CI based on the maximum likelihood estimation of parameter c. (c) Litter sizes as a function of the proportion of the litter that was sired by a $+/t$ male. The dotted line and the white shaded area illustrate GLM predictions including the 95% CI. In all panels, the surface area of the dots represents the number of observations for a given x, y combination. Black color is used to depict mating crosses that involve +/+ females, red colors are used for mating crosses with $+/t$ females.

Mini-Chapter 5

The Effect of Polyandry on a Distorter System with Differential Viabilities in the Sexes

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Abstract

The presence of selfish genetic elements can have fatal consequences for populations that harbor them. In the well known *t* haplotype in wild house mice, large proportions of the population die from t/t recessive lethal effects. Due to strong advantages at the gamete level (drive), *t* haplotypes nevertheless occur at substantial frequencies. The stable presence of a lethal is not the only effect of the *t*. It also distorts the fate of mutations that differentially affect male and female survival and reproduction (such as in sexual conflict), by giving male selective effects a strong advantage over female selective effects. In a recent study, we proposed polyandry as a potential counterstrategy against *t* deleterious effects. Here, we show that (1) the efficiency of polyandry in reducing the t frequency strongly depends on the selective context and (2) polyandry helps to reduce male-biased leverage in sex dependent selection.

Keywords *t* haplotype, intragenomic conflict, sexually antagonistic effects, segregation distortion, overdominance

The 'fair' Mendelian 50:50 ratio of chromosomal segregation during meiosis is an important ingredient to our understanding of evolution. Yet an increasing number of selfish genetic elements are described that systematically deviate from Mendelian expectations (Burt and Trivers, 2006). By distorting Mendelian inheritance ratios in their favor, selfish genetic elements spread in populations despite fatal fitness consequences for their hosts. One of the best known selfish genetic elements is the t haplotype in house mice (*Mus domesticus*). The t haplotype is a variant of mouse chromosome 17 and comprises a whole complex of genes that is protected from recombination through inversions (Silver, 1993). Male +/t heterozygotes transmit it to up to 90% of their progeny (Silver, 1993). This deviation from the typical 50% inheritance ratio is called drive (τ). The strong advantage on a gamete level is opposed by negative fitness effects on an individual level: most t haplotype variants carry recessive lethal alleles. As a result, t/t homozygotes die early during embryogenesis (Hartl, 1970).

Drive is exclusive to one sex in most known cases. In the t haplotype, it is exclusive to males. It follows that a mutation changing the reproductive value of a male will be selected more strongly than a mutation with identical effects in females (Hartl, 1970; Burt and Trivers, 2006).

Recently, we studied the effects of polyandry on expected distorter frequencies, allowing viability selection to differ between males and females (Manser et al., 2011). We showed that polyandry can lead to substantial reductions in expected mean t frequencies, as t carrying males do typically worse in sperm competition (Haig and Bergstrom, 1995). Here, we investigate more systematically under which combinations of viability polyandry is most effective in reducing the frequency of the t. Parameter choices were inspired by measurements from our wild house mouse population (i.e. scenario II).

Sex dependent selection without drive and polyandry Figures 1A–D illustrate the influuence of sex dependent selection, drive and polyandry on equilibrium t frequencies in an infinite, well-mixed population. These figures are based on the model presented in Manser et al. (2011). The results in Figure 1A,B are identical to the analytical solutions of Hartl (1970). Sex dependent selection coefficients s_i, where i defines sex, denote relative viability differences between +/+ and +/t individuals. Relative viability of +/t individuals is therefore $w_{+/t(i)} = 1 - s_i$ relative to a value of one for +/+ homozygotes ($w_{+/t(i)} = 1$). t/t homozygotes are lethal in both sexes (i.e. $w_{t/t(i)=0}$). Without drive ($\tau = 0.5$, see Figure 1A) t haplotypes only occur at equilibrium when overdominant at least in one sex. Note that without drive, equilibrium t

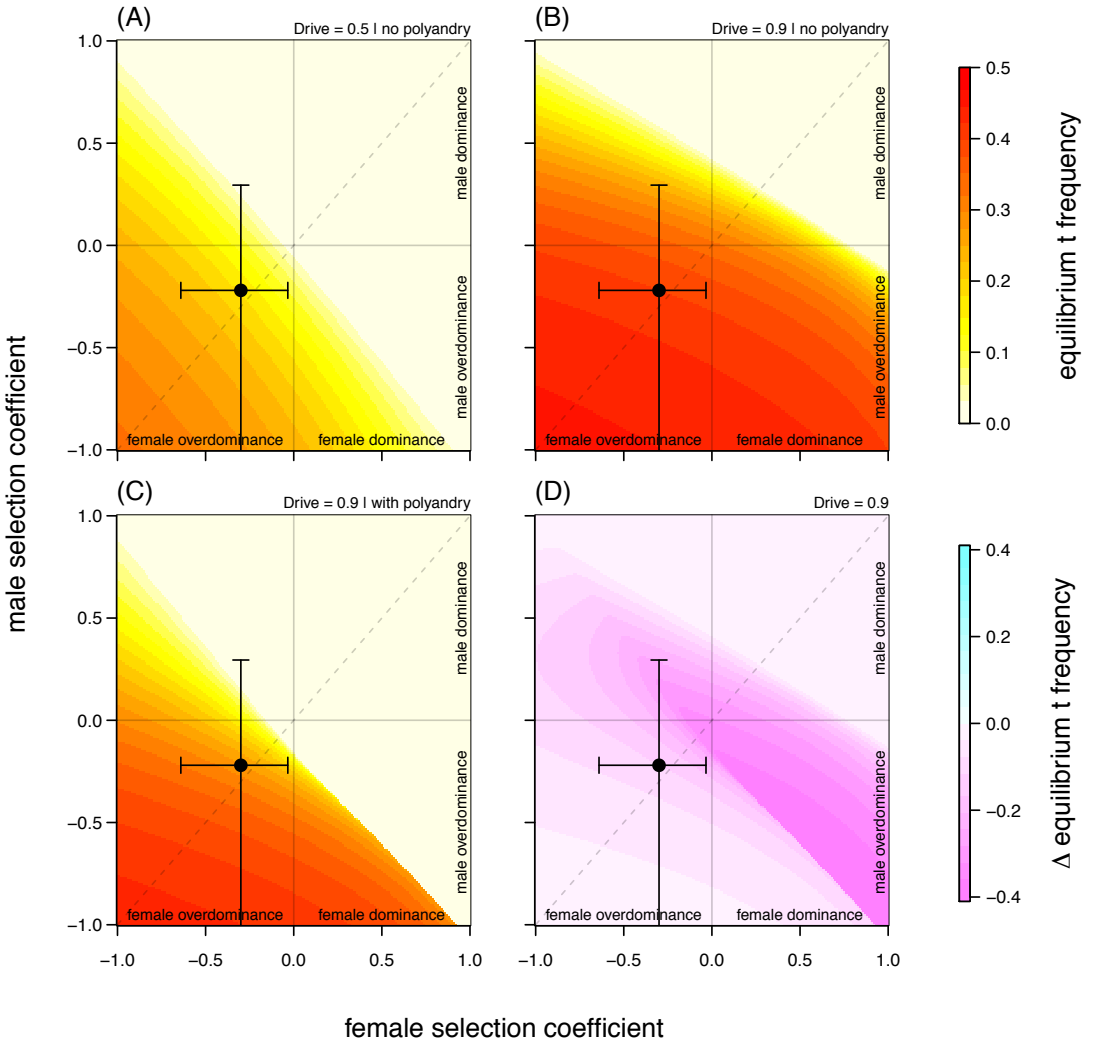

Figure 5.1. Equilibrium t frequencies $\hat{p}_t$ as a function of male and female relative selection coefficient s_i (A) without drive and polyandry ($\tau = 0.5$), (B) with drive without polyandry ($\tau = 0.9$) and (C) with drive and polyandry ($\tau = 0.9, \psi = 0.6, c = 0.88$). Figure (D) shows the difference in equilibrium frequency between (B) and (C) $\Delta\hat{p}_t$. The upper right quadrant represents cases of incomplete dominance, the lower left quadrant cases of overdominance in both sexes. The upper left and lower right quadrants capture sexually antagqonistic alleles. Black cirqcles indicate selection coefficients observed in our study population (including 95% CI bands).

frequencies $\hat{p}_t$ are symmetrical with respect to the diagonal (dashed line), indicating that selection has the same effect on $\hat{p}_t$, irrespective of whether it occurs in males or in females.

Sex dependent selection with drive Figure 1B shows the same relationship for the drive levels observed in our study population ($\tau = 0.9$). There are two important changes: (1) As expected, drive leads to a general increase $\hat{p}_t$ for given selection levels. (2) Equilibrium t frequencies are no longer symmetrical with respect to the diagonal (dashed line). Now, a mutation with a given set of selection coefficients $\{s_f = x, s_m = y\}$ typically results in a different than a mutation with opposite effects in the sexes . More precisely, male selective effects dominate the evolutionary outcome, as they are enforced by drive. This asymmetry has interesting implications for sexual conflict (see below).

Adding polyandry Figure 1C analyzes the effects of polyandry on $\hat{p}_t$. Parameter settings are based on the intermediate polyandry scenario II of the original publication, which assumes a fraction $\psi = 0.6$ of females mating twice, and $+/t$ heterozygote sperm competitiveness levels of $c = 0.88$. In this model, $+/t$ males are less likely to successfully fertilize eggs in sperm competition with $+/+$ males due to reductions in sperm quantity (as a consequence of drive τ) and sperm quality (described by the sperm competitiveness parameter c). The difference in equilibrium t frequency to the model without female multiple mating is shown in Figure 1D. Clearly, the decrease in t frequency due to polyandry is not uniformly distributed on the continuum of selection coefficients s_i. In cases of female dominance and male overdominance, multiple mating is more efficient in removing the t allele from the population than in others. We think the main reason for this is the fact that polyandry is a frequency dependent process. Sperm competition can only play a role in cases where a female mates with a male of each genotype ($+/+$ and $+/t$). The probability for such a mating combination is highest if about half of the male population carry a t (hence $p_t \simeq 0.25$). Consequently, the combination of male overdominance, which keeps male t frequency at these optimal high levels, and female dominance, which reduces t frequency independent of sperm competition, creates the strongest impact on $\hat{p}_t$.

Empirical data Data on the sex specific fitness consequences from wild house mouse populations, especially on the $+/t$ heterozygote fitness effects of the t haplotype, are still scarce and contradictory (e.g. Dunn et al. (1958); Carroll et al. (2004)). In Manser et al. (2011), we quantified the effect of the t haplotype on male and female survival into adulthood based on a free-living house mouse population near Zurich. In

agreement with Dunn et al. (1958), we found a survival advantage of $+/t$ heterozygotes in both sexes. The estimated selection coefficients were within a range where polyandry is not particularly efficient against the t (see also Fig. 1): $s_f = -0.39$ (95% CI: $[-0.64, -0.03]$ and $s_m = -0.22$ (95% CI: $[-1.12, 0.29]$. Because of the low confidence in the male estimate, we used $s_m = 0$ in the original paper.

Drive and sexual conflict Males and females have distinctive roles in reproduction. A situation where a trait expressed in both sexes has different fitness optima in males and females is referred to as intralocus sexual conflict or sexually antagonistic selection (Rice, 1984). It has been argued that drive, because it is usually sex-biased, can have consequences for intralocus sexual conflict (Burt and Trivers, 2006). A sexually antagonistic gene giving males a selection advantage (e.g. 10%) and females a disadvantage (e.g. 15%) may be positively selected with the help of drive, even if the net effect over both sexes is negative. We did not find any indication of sexual conflict in the trait measured here (survival to sexual maturity). However, Chippindale et al. (2001) suggested that early developmental effects are unlikely to be sex biased, as the fitness objectives for both sexes are likely to coincide. For future studies, it would thus be interesting to investigate whether we find signs of intralocus conflict in adult traits such as fertility or reproductive success, where gender roles diverge. Our model suggests that polyandry considerably reduces the asymmetry induced by drive (Fig. 1C). Polyandry may therefore not only be a successful female mating strategy against the lethal effects of the t, but thereby also help to reduce a male-biased leverage in sexual conflict.

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Chapter 6

Frequency, Heritability, and Fitness Consequences of Polyandry in a Natural House Mouse Population

Andri Manser, Barbara König, Anna K. Lindholm

Abstract

The female tendency to mate with more than one male (termed polyandry) is ubiquitous across the animal kingdom. Despite extensive research efforts, we understand little of the forces that drive the evolution of polyandry, particularly under natural conditions. Here, we measured the frequency, heritability, and fitness consequences polyandry in a wild population of house mice *(Mus musculus domesticus)* that has been intensively monitored for over 10 years. Parentage analysis on litters born between 2006 and summer 2010 revealed that 47% of the litters were sired by multiple male (defined as genetic polyandry). Based on a detailed population pedigree, we first quantified the importance of genetic and environmental factors for the occurrence of genetic polyandry using an animal model framework. We found that the occurrence of multiple paternity was mainly determined by environmental factors such as population density. Intrinsic factors such as female identity or heritable genetic variation had little influence on trait expression ($h^2 < 0.01$), suggesting that females exercise little control over mating rates. In a second step, we quantified the fitness consequences of genetic polyandry and sperm competition in the two sexes. It has been hypothesised that polyandry and subsequent sperm competition may help female avoid indirect fitness costs related to meiotic drive elements. The presence of a well-known drive gene (called *t* haplotype) throughout the observation period allowed us to test key predictions related to this drive-hypothesis. Surprisingly, we found that neither genetic polyandry rates nor the *t* haplotype affected total offspring number in females. In males, on the other hand, reproductive success was negatively affected by intensity of sperm competition. In line with the drive-hypothesis, we found that sperm competition was particularly detrimental to the reproductive output drive-carrying males, making this the first direct evidence of polyandry-related drive suppression under natural conditions.

Keywords polyandry, multiple paternity, heritability, selection gradient, sperm competition, house mouse, *t* haplotype

6.1 Introduction

The number of mating partners is an important determinant of an organism's reproductive success. In the mate-limited sex, usually the males, it is relatively straightforward to see how mating with several partners (termed polygyny) translates into larger reproductive output. In the mate-unlimited sex, usually the females, the benefits of several mating partners in (termed polyandry) are far less obvious (Bateman, 1948). The problem is accentuated by the fact that every mating is typically associated with costs, e.g. energy expenditure, physical damage, or exposure to sexually transmitted diseases (e.g. Chapman et al. (1995); Arnqvist and Nilsson (2000)). Yet empirical evidence suggests that polyandry is common across the animal kingdom. The forces that drive the evolution of polyandry have become a central topic in evolutionary research (e.g. Pizzari and Wedell (2013)).

The occurence of polyandry A large body of empirical work indicates that polyandry is widespread across a wide range of taxa, suggesting that female multiple mating is the rule rather than the exception (Birkhead and Møller, 1998). The estimation of polyandry rates in natural populations has been greatly facilitated by the possibility of molecular paternity assignment (Bretman and Tregenza, 2005). Reports of mixed paternity in clutches and litters based on genetic markers are numerous and often include species that were previously thought to be strictly monogamous based on behavioural observations. The indirect inference of female mating behaviour through genetic analysis of a female's offspring is powerful, because it does not require direct behavioural observations. However, it is important to note that measuring multiple paternity rates will result in an underestimation of the actual multiple mating rates, because not every male a female mates necessarily succeeds in fertilization. It is hence important to distinguish between multiple mating (henceforth termed (behavioural) polyandry) and multiple paternity (henceforth termed genetic polyandry). Several methods have been used to infer behavioural polyandry rates from genetic polyandry (Neff and Pitcher, 2002; Dean et al., 2006; Manser et al., 2011; Simmons et al., 2007).

The benefits of polyandry Numerous hypotheses have been advanced to explain the overall selective benefit of polyandry in spite of the above mentioned mating costs (for a review see, for example, Jennions and Petrie (2000)). Among the many adaptive explanations for polyandry, it has been proposed that mating with several males may help females avoid direct fitness costs related to drive elements Haig and Bergstrom (1995); Wedell (2013). Drive elements define stretches of DNA that ma-

nipulate gamete production in their own favour, typically by systematically killing rival sperm within males (Burt and Trivers, 2006). Due to the demise of a large fraction of its sperm, drive-carrying males are typically strongly compromised in sperm competition (Price et al., 2008a; Wedell, 2013).

The heritability of polyandry Understanding the costs and benefits (i.e. selection) of polyandry is a necessary, but not a sufficient requirement for an adaptive explanation of polyandry (or monandry). Ongoing evolution on female mating number also requires the presence of genetic variance (h^2) for the trait. For example, due to the absence of heritable variation, polyandry may persist in a population despite being costly to females. Moreover, testing for the presence of heritable variation appears particularly important here, as polyandry has been regarded as a mere by-product of a general tendency of a mate with partners encountered (**?**). In this case, we expect trait variation to be mainly determined by environmental variables such as mate availability. Despite the importance for the understanding of polyandry evolution, only a handful of studies have estimated additive genetic variance for the trait, limited to few taxa. A number of laboratory studies on insects have found considerable broad-sense heritability for female re-mating rates, but additional studies that allowed for the partitioning of the total genetic variance between dams and sires suggested that these estimates were dominated by maternal effects. Accordingly, narrow-sense heritability estimates were low ($h^2 < 0.1$, reviewed in Evans and Simmons (2008)). These low estimates are in line with the three studies that have estimated narrow-sense heritability in vertebrate taxa (in guppies: $h^2 = 0.11$ (Evans and Gasparini, 2013); in North American red squirrels: $h^2 < 0.01$ (McFarlane et al., 2011); in song-sparrows: $h^2 = 0.12$ (Reid et al., 2011)). Note that heritability estimates are dependent on the context in which they are measured. Under natural conditions, environmental effects typically have larger effects on the trait of interest, resulting in smaller heritability estimates compared to controlled laboratory conditions (Postma, 2014). Only two of the above mentioned studies were measured in a natural context. Yet, to understand the evolution of polyandry, it seems important to measure the genetic basis of phenotypic variation in the specific context in which selection on the trait actually acts, that is in natural populations.

Polyandry and gene drive in house mice In this study, we provide an in-depth analysis of genetic polyandry and its fitness consequences under natural conditions in wild house mice (*Mus musculus domesticus*). To this end, we analyse data from a wild mouse population outside Zurich that has been intensively monitored for over 10 years.

Several aspects make house mice, and the study population in particular, an ideal system for the study of polyandry. Firstly, house mice have been shown to be actively polyandrous, and often produce litters sired by more than one male both under laboratory and natural conditions (Dean et al., 2006; Firman and Simmons, 2008c; Manser et al., 2011; Auclair et al., 2014; Thonhauser et al., 2014). Secondly, the study population harboured a well-known drive system called the *t* haplotype (Manser et al., 2011; Lindholm et al., 2013). This circumstance allowed us to test specific *a priori* predictions related to polyandry and gene drive. The *t* haplotype is a well-known variant of mouse chromosome 17 that distorts Mendelian inheritance ratios in males (termed gene drive). As a result, the *t* is transmitted to about 90% of the offspring in heterozygous $+/t$ males (Lindholm et al., 2013). Transmission ratios in females, on the other hand, are perfectly Mendelian. As is the case for most known *t* haplotype variants (Klein et al., 1984), the *t* haplotype in our study population has detrimental fitness consequences for individual carriers. Due to recessive lethal mutations, t/t homozygote individuals perish *in utero*. As a direct result of recessive lethality and drive, $+/t$ females suffer from a dramatic, 40% litter size reduction in monogamous matings with a $+/t$ male (Lindholm et al., 2013). In line with the polyandry hypothesis outlined above, recent experimental work has shown that $+/t$ males from our study population perform poorly in sperm competition, only fertilising about 13% of the offspring when competing with a $+/+$ male (Sutter and Lindholm, 2015). As a consequence, multiple mating and subsequent sperm competition disadvantage of $+/t$ males helped $+/t$ females to avoid *t*-related litter losses (Sutter and Lindholm, 2015).

Here, we measured the frequency, heritability and fitness consequences of genetic polyandry in a wild population of house mice. Due to the expected asymmetries in the polyandry-related fitness pay-offs between $+/t$ and $+/+$ females, we expected elevated polyandry rates in $+/t$ females. The study is divided in two parts. In the first part, we quantify the importance of genetic and non-genetic factors for the occurrence of genetic polyandry. A detailed population pedigree allowed us to estimate additive genetic variation for the trait (V_A). In the second part, we measure the fitness consequences of polyandry and the *t* haplotype in the two sexes.

6.2 Material and Methods

Study Population and Data Collection

The data was collected in a free-living population of house mice inhabiting a 72 m^2 farm building near Zurich (König and Lindholm (2012) for a detailed description). The population was founded in 2002 by 12 individuals caught from the surrounding area and has been intensively monitored ever since. Mice are provided with nesting opportunities (40 artificial nest boxes), nesting material and *ad libitum* food and water. Vertical metal plates, bricks, plastic tubes, and branches structure the environment and provide additional hiding places. Small openings in the walls and roof allow mice to freely leave and enter the population. None of the avian and mammalian predators are able to access the building, but predators are regularly observed in close vicinity. The population set-up is thought to closely resemble the natural habitat of house mice, since mice typically live commensally with humans, thus in places where food and nesting opportunities are available in abundance (Berry et al., 2008).

Monitoring Reproduction Reproductive activity of the mice has been closely monitored since the population was set up. Nest boxes are checked for newly born litters on a weekly basis. (Re-)capturing of mice at subadult and adult stage (see below) suggest that we detect the majority of newborns in the population using this method. Newly detected litters are documented and age determined based on morphological characteristics (König and Lindholm, 2012). At ca. 13 days of age, before pups begin to be mobile, tissue samples are collected from every pup that survived until that stage for subsequent genetic analysis (see below). About every 7 weeks, the entire population is captured, sexed, and individually marked, allowing us to estimate the overall density in the population. For more details on population monitoring, see König and Lindholm (2012). For the purpose of this study, we have focused on 3,127 pups born in 1,015 litters from 279 females that were born between January 2006 and July 2010.

Genetic analyses Parentage of all sampled pups was assigned using 25 polymorphic microsatellite markers distributed across the mouse genome (see Auclair et al. (2014) for marker and PCR details). Parentage analyses were performed using CERVUS 3.0 (Kalinowski et al., 2007). We assembled candidate mother lists for each offspring based on those females that were present within two days of the offspring's estimated birthdate. Candidate father lists included all males present at the estimated time of conception. As the gestation period in mice is typically 19 days (Berry et al., 2008) but

is extended following postpartum fertilisation (Brambell, 1937) we defined the time of conception as 17–26 days before birth. Parentage analyses were performed for each year separately. We used an error rate of 0.01 based on the frequency of scoring differences in repeat PCR amplifications of all loci for 100 individuals (measured at 0.006). The proportion of loci typed was 0.99. We generated critical delta values in simulations of 100,000 offspring and a sampling frequency of 0.9 for mothers and fathers. Parentage assignments were only accepted at a 95% level of confidence and only when no more than one mismatching allele occurred between parent and offspring.

Based on parentage assignment, a full pedigree is available for the entire population. The *t* genotype of an individual was identified on the basis of a microsatellite marker (*Hba-ps4*) that contains a *t*-haplotype specific 13 base-pair insertion (Schimenti and Hammer, 1990).

Explaining Phenotypic Variance in Genetic Polyandry

In the first part, we analysed the effect of environmental and genetic factors on the probability that a litter was sired by more than one father (genetic polyandry) using a generalized animal model. Generalized animal models are a specific type of a generalized linear mixed effects models (GLMM) that use a pairwise relatedness matrix (derived from a pedigree) as a random effect variable (Wilson et al., 2010). This approach allowed us to specifically estimate the additive genetic variance (V_A) of genetic polyandry (Lynch and Walsh, 1998; Wilson et al., 2010). Fitting an animal model for non-normally distributed traits can be challenging or impossible using conventional (restricted) maximum-likelihood methods. We thus analysed our model with a Bayesian framework, using the Markov chain Monte Carlo algorithm as implemented in the R package MCMCglmm (Hadfield et al., 2010a; R Core Team, 2015).

Overall model structure Although the number of fathers per litter varied between 1 and 4 (see Figure S6.1), we treated a female's polyandrous tendency as a binary response trait for the purposes of this section. Accordingly, each litter i was categorized either as $p_i = 0$ if sired by one male or $p_i = 1$ if sired by more than one male, where i corresponds to the i-th observation/litter. Litters of size one were excluded from the analysis, because multiple paternity is not possible in these cases. Owing to the binary nature of the response variable p_i, we estimated effect sizes of several random and fixed predictor variables using a logit-transformation (since p_i cannot exceed 0 and 1) and a binomial error distribution.

Random effect structure Two random effect variables were included in the model, the random additive genetic effect (V_A) and maternal identity (V_{PE} for permanent environment). To estimate the amount of trait variation that can be attributed to heritable genetic variation among females (V_A) we used the parentage analysis (as described above) to construct a pairwise relatedness matrix of all 225 females in the data set. From the entire pedigree, we removed all non-informative animals (animals that did not reproduce and/or were not responsible for a link between two informative animals) using the `prunePed` function in the R package `MCMCglmm` (Hadfield et al., 2010b). The remaining pedigree contained a total 451 individuals, of which 41 individuals (9%) were treated as founders due to unknown maternal and paternal links. The average depth of the pedigree was 8.44, the maximum depth equalled 17. About 50% of the individuals had an inbreeding coefficient greater than 0, and the average degree of inbreeding was 0.065. The mean pairwise relatedness was 0.068, while about 20% of individuals were greater than 0.125. Pedigree summary statistics were produced with the help of the `Pedantics` package in R (Morrissey and Wilson, 2010).

Several females in the data set reproduced more than once. We accounted for such repeated female reproduction by fitting female identity as an additional random effect variable. This allowed us to test for potential, systematic differences in polyandry rates among females. In quantitative genetics studies, this variance component is usually termed V_{PE} (for permanent environment).

Fixed effects Additional to the random effects variables, we fitted a number of additional predictors as fixed explanatory variables. In a full model, we investigated the effect of female t genotype ($+/+$ and $+/t$), adult population size and average monthly temperature at the time when the litter was born, as well as the size of the litter (without interactions). Note that the inclusion of litter size as an explanatory variable is of crucial importance here, as we expect a higher probability of genetic polyandry in larger litters based on chance alone (because of the reduced sampling error in larger litters).

Implementation details Bayesian analyses require the specification of prior probability distributions for all random and fixed predictors used in the model. We used relatively uninformative priors for both fixed effects (normally distributed with a mean of 0 and a variance of 10^8) and random effects (inverse Wishart distributed, with variances set to 1 and degree of belief ν of 1). Because estimated variance components V_A and V_E were close to zero, we used parameter expansion to ensure proper mixing of the posterior chains. Model outcomes were robust with regard to the (reasonable) choice

of prior distribution. Note that residual variance V_R cannot be estimated in binary models (Hadfield, 2012; Nakagawa and Schielzeth, 2010; Postma et al., 2011) and were therefore set to a fixed, arbitrary value of 1. It is important to mention that, while the arbitrary choice of V_R does affect absolute estimates of the other variance components V_A and V_{PE}, it has only minor effects on the *relative* magnitude of the three variance components. Hence, the choice of did not affect our heritability estimates. We run all models for 10^6 iterations, with a burn-in of 5,000 and a thinning interval of 3,000 to avoid autocorrelation among the samples from the posterior distribution. After running a full-model including all fixed and random variables, we removed non-significant fixed factors in a stepwise manner.

Calculating heritability Heritability h^2 is defined as the proportion of phenotypic variance (V_P) that is accounted for by additive genetic variance V_G). We were interested in the heritability of a female's *propensity* for genetic polyandry. In the model here, the propensity is not estimated on the scale at which the trait was measured (data-scale), but on the underlying logit-scale (latent scale). Accordingly, we used the following expression to calculate the latent-scale heritability of genetic polyandry

$$h^2 = \frac{V_A}{V_A + V_{PE} + V_R + \frac{\pi^2}{3}}, \tag{6.1}$$

where the logistic variance is proportional to $\frac{\pi^2}{3}$ (Nakagawa and Schielzeth, 2010).

Effects of Polyandry and t Haplotype on Reproductive Output

In a second part, we measured the effect of genetic polyandry on an individual's reproductive output (fitness). To this end, we measured the selection gradients, i.e. the statistical relationship between an individual's genetic polyandry rates and its reproductive output. Genetic polyandry, by inviting sperm competition, is likely to affect the reproductive output in males and females. We thus measured selection gradients in both sexes. Equipped with specific *a priori* predictions from laboratory experiments (see below), we were particularly interested in possible interactions of male and female selection gradients with t genotype.

Explaining variation female reproductive success We used the total number of offspring at day 13 (time of genetic sampling) produced by a given female during the observation period as a measure of female fitness. We consider this measure a good approximation of lifetime reproduction, although the tenure of some females

overlapped with the observation period (right or left-censored). Total offspring number was analysed as a function of multiple mating rate (the focal trait), the t genotype of the female ($+/t$ or $+/+$), the number of reproductive events (litters), and their two-way interactions using a generalized linear model with a Poisson error-distribution and a logarithmic link-function. As a predictor value for an individual female's multiple mating rate, we used the best unbiased linear predictors (*BLUP*) for each individual as derived by the animal model (see above). We performed a systematic model selection based on AIC values using the `dredge` function from the `MuMIn` package in R.

Explaining variation in male reproductive success We investigated whether sperm competition among males, induced by polyandry, affected male reproductive output. Once more, we used the total number of offspring (at day 13) sired by a given male during the observation period as a proxy for fitness. Total reproduction was again analysed as a function of the average number of other sperm competitors (that sired at least one offspring), t genotype status, total number of reproductive events (litters with at least one sired offspring), and their two-way interactions using a GLM assuming a quasi-Poisson distribution and a logarithmic link function. Model selection was again performed systematically using the `dredge` function based on $qAIC$-values.

Additional fitness measures We ran additional analyses to investigate the effect of polyandry on reproductive output, this time using litter instead of individuals as the focal unit. Accordingly, we fitted the number of offspring sired as a function of the number of sperm competitors, the genotype of the mother/father, and their interaction. To account for the fact that the same individuals often sired offspring across multiple litters, we fitted individual identity as a random factor. To assess whether potential differences in reproductive output between $+/t$ and $+/+$ individuals were attributable to differences in mating success, we further compared the total number of reproductive events during the observation period as a function of t genotype for both sexes.

6.3 Results

The population context Supplementary Figures S6.1–S6.3 give a descriptive overview of the population context in the time period considered in this study. Reproductive activity follows a strong seasonal pattern, as the majority of litters are born during the warmer seasons between spring and autumn (König and Lindholm, 2012).

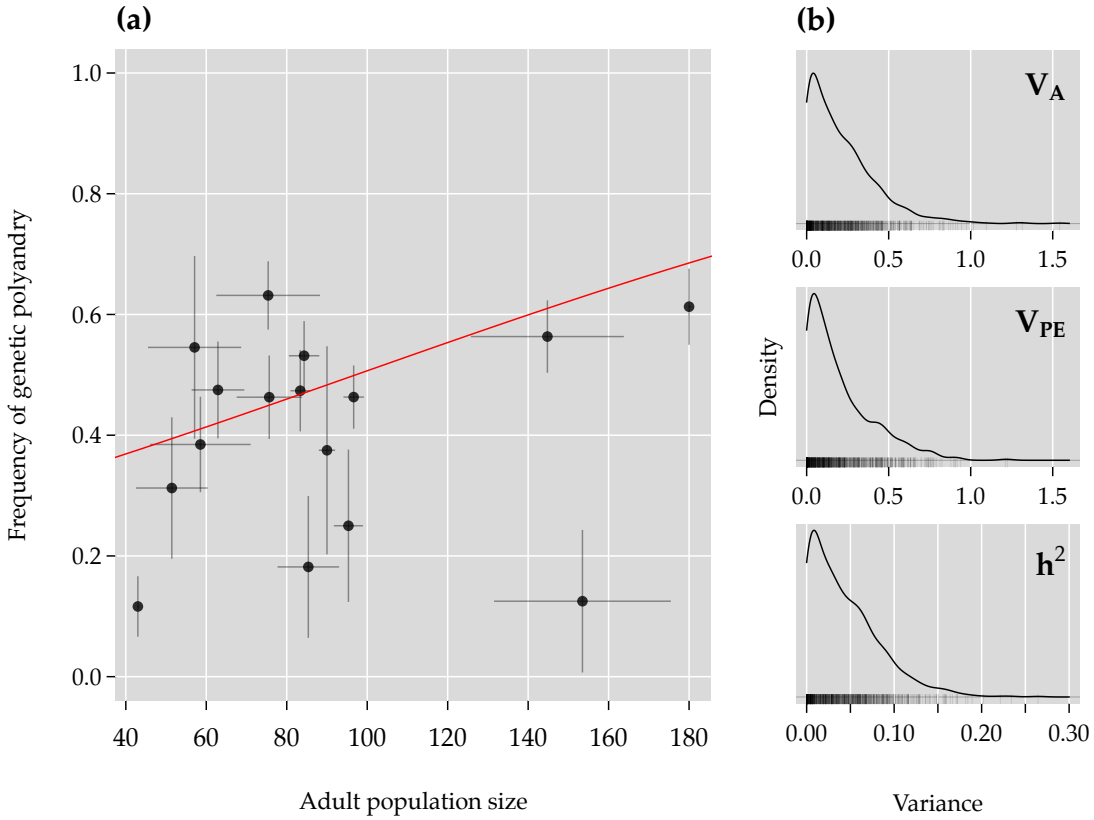

Figure 6.1. (a) Positive relationship between the frequency of polyandry and adult population density as predicted by the generalized animal model (red line). The raw data was grouped in quarter-year time intervals, with vertical lines indicating binomial standard errors in genetic polyandry rates and horizontal lines standard errors in adult population size. (b) The posterior probability density distributions for the two variance components considered in the generalized animal model (additive genetic variance V_A, permanent environmental variance V_PE) and the resulting density distribution for the heritability h^2.

The number of identified genetic fathers per litter varied considerably across the observation period, with the number of (successful) sires ranging between one (genetic monogamy) and four (genetic polyandry). Overall, adult population size increased over the four-year observation period. As reported previously Manser et al. (2011), the proportion of t haplotype carriers both among newborns as well as reproductively active adults dropped considerably during the observation period. Interestingly, the t frequency among reproductively active dams was typically higher than among reproductively active sires, suggesting the presence of a mechanism that reduces the fertilization success of $+/t$.

Explaining phenotypic variance in genetic polyandry Among 682 informative litters (i.e. with a litter size larger than 1) from 225 females born between January 2006 and July 2010, 323 were sired by more than one father (47%). Average monthly temperature and female t genotype had no statistically relevant effect on trait variation, and thus removed by model selection. As expected, owing to the larger detection probability of genetic polyandry in larger litters, we found a positive relationship between litter size on the occurrence of genetic polyandry (posterior slope estimate: 0.26, 95% CI: [0.14, 0.37], $P < 0.001$). Moreover, the occurrence of genetic polyandry increased with adult population size (Fig. 6.1a, posterior slope estimate: 0.87, 95% CI: [0.33, 1.41], $P < 0.01$).

Both female identity and heritable genetic variation explained very little to no variation in the occurrence of genetic polyandry, as estimates for both variance components converged towards zero (Fig. 6.1b). As a consequence, the heritability estimate of genetic polyandry was very low, with a posterior mean and 95% CI of $h^2 = 4.32 \times 10^{-4}$ [0, 0.12] (Fig. 6.1b).

Fitness consequences of genetic polyandry Among the 225 females that reproduced at least once (note that we included here only litters of two or more pups), we found considerable overall reproductive skew. In the 4.5 year considered, females produced on average 3.03 litters (ranging up to 11), resulting on average in 12.41 pups that reach the age of 13 days (maximal reproductive success was 55). Neither of the two variables of interest, a female's genetic polyandry frequency (*BLUP*) or t genotype, significantly affected overall reproductive success (Fig. 6.2a). Accordingly, both variables were removed during model selection, leaving the number of litters as the sole explanatory variable explaining female fitness (effect size estimate: 0.26, $SE = 0.007$, $P < 0.001$). Moreover, $+/t$ and $+/+$ females did not differ in thenumber of litters.

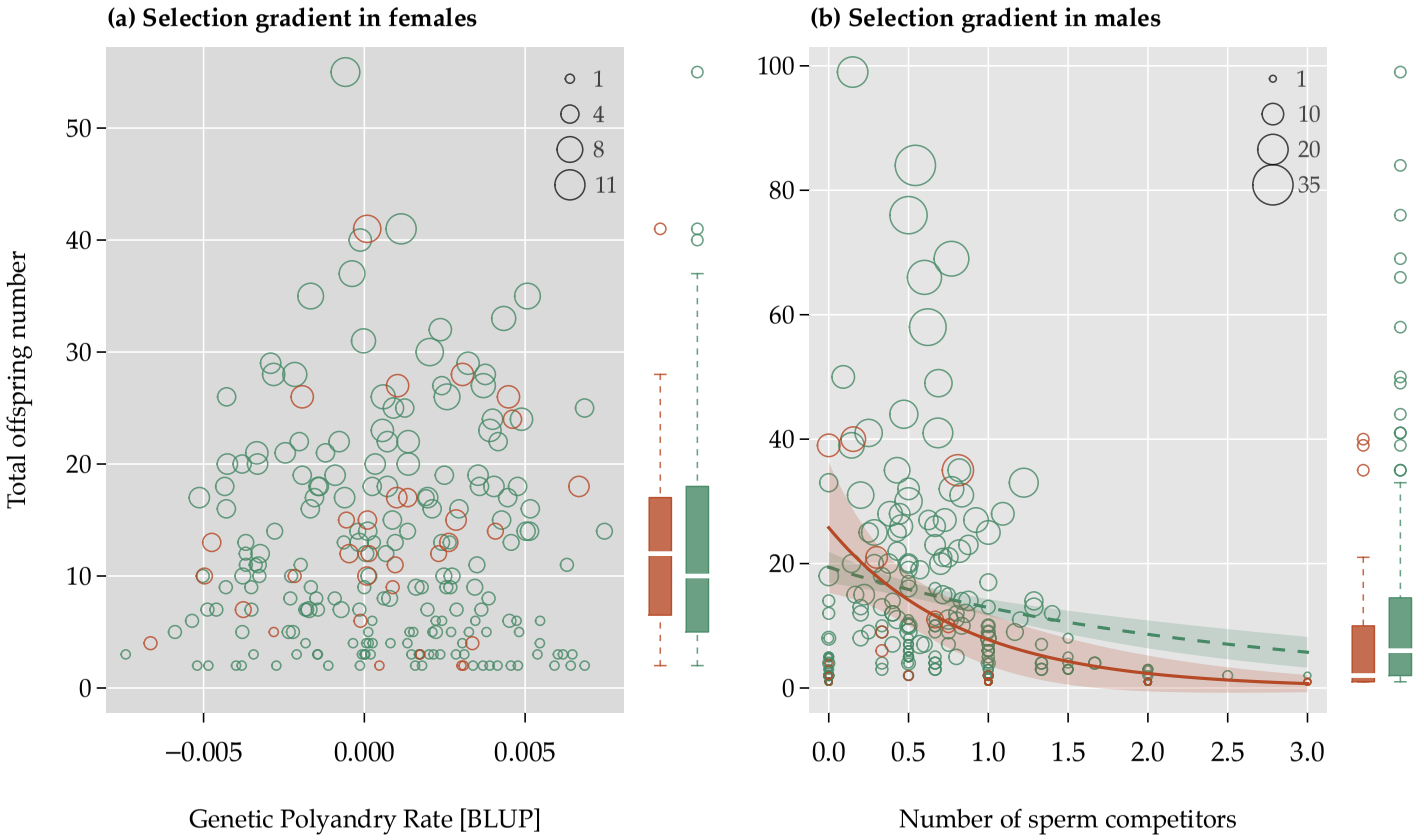

Figure 6.2. Lifetime reproductive success as a function of (a) genetic polyandry rate in females and (b) the number of male sperm competitors (averaged across reproductive events). An individual's t genotype is represented by red for $+/+$ and green for $+/t$ individuals. Dot size is proportional to the number of reproductive events (as indicated by the legends in the top-right corners). Boxplots show the difference in reproductive success as a function of t genotype only. In males, GLM predictions and 95% confidence bands for $+/t$ and $+/+$ males are represented by the red-solid and green-dashed lines, respectively (model predictions are shown for a hypothetical male that reproduced 10 times).

249 males successfully sired at least one offspring during the observation period. On average, they sired 11.32 pups (ranging up to 99) distributed over 4.8 reproductive events (the maximal number of reproductive events was 35). On average, a male had to compete against 0.76 other males, ranging up to 3. The model yielding the lowest $qAIC$ value included all three explanatory variables (number of competing males, number of reproductive events, t genotype) and the two-way interactions between t genotype and number of competing males and between t genotype and number of reproductive events (see Fig. 6.2b for GLM predictions). According to the minimal adequate model, $+/+$ and $+/t$ males do not differ in their total reproductive success if only mating monogamously (difference in intercepts between $+/t$ and $+/+$ males: -0.62, $SE = 0.54$, $P = 0.25$). Both male genotypes were negatively affected by the average number of sperm competitors they encountered during the observation period (slope estimate in $+/+$ males: -1.19, $SE = 0.37$, $P < 0.01$). Importantly, we find a significant interaction of sperm competition intensity with t genotype, with $+/t$ males being significantly stronger affected by the number of sperm competitors (difference in sperm-intensity slopes between $+/t$ and $+/+$ males: -0.79, $SE = 0.38$, $P < 0.05$; also see Fig. S6.4 for an analysis that uses individual litters as the focal unit). As expected, we find that the number of reproductive events increases a males reproductive success (estimate: 0.14, $SE = 0.02$, $P < 0.001$).

6.4 Discussion

In this study, we have investigated the genetic and non-genetic factors that affect the occurrence of genetic polyandry and its fitness consequences in a natural population of house mice. We show that female multiple mating is common in house mice. The overall genetic polyandry rates in the observed 4.5-year period was 47%. We find that the occurrence of multiple paternity is mainly determined by environmental factors such as population density. There is little evidence to suggest that females systematically differ in their polyandry rates, nor that the trait is heritable. We also find no evidence for systematic differences in polyandry rate related to the t haplotype. In females, genetic polyandry rates did not affect the total offspring number, suggesting no or little selection on the trait. In males, on the other hand, reproductive success was negatively affected by number of sperm competitors. In line with *a priori* expectations, we found that sperm competition was particularly detrimental to the reproductive out-

put of t haplotype carrying males. We thus provide first direct evidence that polyandry suppresses gene drive under natural conditions.

Mice are highly polyandrous The genetic polyandry rates of 47% measured here are well in the range of previous estimates in wild house mice, both under laboratory and natural conditions. Laboratory experiments, conducted on mice originating from the population studied here (Manser et al., 2015; Sutter and Lindholm, 2015) and elsewhere (Thonhauser et al., 2013, 2014; Rolland et al., 2003), have reported multiple paternity rates around 30–40%. These laboratory estimates seem representative of the situation in natural populations, where multiple paternity rates were estimated around 20% in North-American and 4–47% in Australian wild house mouse populations (Firman and Simmons, 2008a; Dean et al., 2006). In line with previous studies, we find that genetic polyandry rates increase with population density (Dean et al., 2006). Due to the expected asymmetry of polyandry related fitness benefits for $+/t$ and $+/+$ females, we investigated whether genetic polyandry rates differ as a function of t genotype. In light of the all the results presented here (i.e. no heritability or selection on polyandry), it is probably not surprising that we did not find such systematic differences between $+/t$ and $+/+$ females here.

No signs of individual or genetic variation for genetic polyandry Despite considerable statistical power, owing to a large data-set and the comprehensive pedigree, we found little evidence of heritable variation in a female's tendency to give birth to litters sired by multiple males. Accordingly, we arrived at a very low heritability estimate of $h^2 < 0.01$. This is the first study to estimate additive genetic variance in genetic polyandry rates in wild house mice. Only a handful of heritability estimates of polyandry are available in other taxa (Evans and Simmons, 2008), and even fewer studies have attempted to measure the heritability of polyandry in a natural context (McFarlane et al., 2011; Reid et al., 2011). Yet the overall picture that emerges appears consistent across taxa. Heritability of polyandry is typically low, even if compared to other behavioural traits (Postma, 2014). When heritability is measured under natural conditions, estimates are typically reduced even further. The only previous study that has measured heritability of polyandry under natural conditions in a mammal (North American red squirrel) has reported an estimate that is strikingly similar to the one reported here ($h^2 < 0.01$, McFarlane et al. (2011)). Also note that animal models typically result in lower heritability estimates if compared to more conventional methods (such as parent-offspring regression or full-sib or half-sib designs, Postma (2014)).

There are several potential explanations for the low heritability measured here.

One possibility is that there is considerable additive genetic variation V_A, but this variation is negligible in view of the far greater, other sources of variation (i.e. V_R, V_{PE}). Unfortunately, the non-Gaussian nature of the trait considered does not allow us to derive standardized, and thus comparable, estimates of the additive genetic variance component V_A (because such estimates will always depend on the arbitrary choice of the residual variance V_R, see Methods). Second, the low heritability estimate may result from methodological difficulties to reliably estimate variance components. Note that we were interested in a female's inherent propensity for genetic polyandry on the latent scale. Each individual trait estimate, a female's tendency to give birth to litters sired by multiple fathers, is based on few observations only: the number of litters a female gave birth to. With an average of 3 litters per female, the sampling error is large, potentially making it difficult to derive robust estimates of the behavioural trait of interest. The fact that we detected little evidence for systematic individual differences (measured by V_{PE}), may be a further indication of this inherent sampling problem. Thanks to a high-quality dataset and a state-of-the-art statistical method, we were nevertheless able to derive heritability estimates with relatively narrow confidence bands. It hence seems unlikely that the low heritability estimate can exclusively be attributed to insufficient statistical power. Finally, it is possible that there is in fact only little heritable variation for genetic polyandry in this system. As outlined above, we are not the first study that finds polyandry to be largely determined by non-genetic factors such as population density. The fact that we did not find traces of consistency within females across several reproductive events fit with that conclusion.

No signs of selection on polyandry or t haplotypes in females We find little indication of phenotypic selection on genetic polyandry in females. In other words, a female's propensity for polyandry did not affect overall reproductive success in the observed period. The absence of selection may be a statistical artefact. Note that we have used the best linear predictors (*BLUP*) from the animal model to investigate a possible trait-association with reproductive output. Importantly, the animal model included litter size as an explanatory variable (to account for the lower detection probability of genetic polyandry in smaller litters). Hence, the *BLUP*s were already corrected for litter size, arguably one of the main determinants of a female's reproductive success. In light of this, it is probably not surprising that we do not find any effect of polyandry on overall reproduction. Alternatively, we have analysed the effect of *average* female polyandry rates on reproduction, hence using a predictor variable that is not corrected for litter size. In this case, we find a statistically relevant, positive relationship with

reproductive output. However, it is again difficult to assess how much of this positive relationship is biologically relevant, and how much can be attributed to the lower detection probability of genetic polyandry in smaller litters. This is a common problem of studies that analyse the fitness consequences of mating partner number (e.g. Gerlach et al. (2012)) and, to our knowledge, there is no straight-forward solution.

A priori, we expected selection gradients on polyandry to differ between females of different t genotype. Laboratory studies on mice caught in the population studied here have shown that $+/t$ haplotype carrying males are strongly compromised in sperm competition (Sutter and Lindholm (2015), Chapter 4). We were able to confirm this effect under natural conditions here (see below). As a result of the reduced fertilization success of $+/t$ males, we expected $+/t$ to avoid substantial litter losses due to t/t homozygote lethality. Wild-type $+/+$ females, on the other hand, do not suffer from t related litter losses irrespective of their polyandry rates. The prediction of a t-genotype-specific relationship between polyandry and litter size was corroborated in the above mentioned laboratory experiments (Sutter and Lindholm, 2015; Manser et al., 2015): $+/t$ females suffer from marked litter losses of up to 50% when mating with a $+/t$ only; losses that are largely avoided if the female mates with multiple males (Sutter and Lindholm, 2015). We were not able to detect such t-related effects on female fitness under natural conditions here. In fact, not even $+/t$ females that had their litters sired by $+/t$ males only showed a reduction in litter sizes compared to the population average. Note that the statistical biases discussed above are an unlikely explanation for the absence of an interaction effect here, as they affect $+/t$ and $+/+$ females in the similar ways. It is possible that a larger number of $+/t$ females suffered complete litter failure (which could not be quantified here). However, we did not find systematic differences in the overall number of reproductive events between the two genotypes. An important difference to the laboratory studies is the fact that our measure of reproductive success here includes pup mortality (as our analysis only analysed pups that survived until 13 days of age). Previous work suggests that pup mortality rates in the study population are considerable, largely caused by infanticide (Auclair et al., 2014). Because larger litters require more provisioning and protection, it is conceivable that such litters suffer from a increased pup mortality. In this case, systematic litter size differences between $+/t$ and $+/+$ females at birth may be levelled out by differential pup survival rates. The question whether t genotype and litter size at birth influence affect pup survival is unknown and deserves further investigation. In summary, in spite of a strong reproductive skew and strong t-related fitness effects

in laboratory studies, we found no signs of selection on polyandry, the t haplotype or their interaction in females.

Sperm competition and t haplotypes affect fitness in males In contrast to females, we find that polyandry and subsequent sperm competition strongly affected male fitness. Accordingly, males that often had to compete against other males in sperm competition saw a reduction in their overall reproductive output. Moreover, and in line with laboratory experiments ((Sutter and Lindholm, 2015), chapter 3 and 4), we show that $+/t$ males are particularly compromised by sperm competition. To our knowledge, this is the first report of drive-related sperm competitive disadvantage in a natural context in any drive system. There are several reasons to assume that this effect is even stronger than reported here. First and most importantly, we are likely to miss a considerable fraction of cases here where $+/t$ did not manage to fertilize any eggs despite mating with a given female. Note that the number of cases missed due to compete fertilisation failure may be substantial. Under controlled laboratory conditions in which females mated with two males during a single oestrus cycle, $+/t$ males of the population studied here fertilized as little as 13% of egg cells when competing against $+/+$ males (Sutter and Lindholm, 2015). Given the average litter size observed here (3.08), the probability of complete +/t male fertilization failure is given by $(1-0.13)^3$ which is equivalent to 65%! Second, we did not account for the t genotype of rival males here. Our estimate is thus likely to include cases where $+/t$ males competed against other $+/t$ males, a situation where t sperm disadvantage does not play a role. However, the fraction of $+/t$ males among fathers was relatively low throughout the observation period (Fig. S6.2). It is thus reasonable to assume that +/t competed against $+/+$ males in the vast majority of cases. In any case, the fact that we were able to detect traces of t-related sperm competitive disadvantage at the level of overall reproduction despite these severe restrictions, underlines the strength of the effect.

Can polyandry account for the observed t frequency dynamics? Using a theoretical modelling approach, we have previously demonstrated that polyandry may play an important role in the observed t frequency decrease in our study population (Manser et al., 2011). In line with this hypothesis, we find here that $+/t$ and $+/+$ males significantly differ in their reproductive success and that the sperm competitive disadvantage of $+/t$ males contributes to the observed difference in total reproduction between $+/t$ and $+/+$ males. However, due to the above mentioned problems to reliably quantify the sperm competition effect in its *entirety*, we still do not know whether

polyandry and sperm competition alone are *sufficient* to explain the overall decrease in t frequency. Intriguingly, Fig. S6.2 shows that the fraction of $+/t$ males among successfully reproducing males is typically smaller than the fraction of $+/t$ females among reproducing females (this gap is particularly evident in 2007). As we have seen above, polyandry and sperm competition are certainly a viable explanation for this gap (as $+/t$ will often not manage to sire offspring, see above). However, there are alternative explanations to explain reduced $+/t$ fertilization success, for example differences in mating success or survival. Survival differences can be ruled out here: $+/t$ and $+/+$ males do not differ in their survival rates in our study population (Manser et al., 2011). Focusing on the years 2004 and 2005, Lindholm et al. (2013) have suggested that $+/t$ females avoid $+/t$ males *before* mating, but they could not categorically rule out a postcopulatory contribution to the fertilisation bias. As outlined above, disentangling pre- and postcopulatory process represents a major challenge, as we only have information on an individual's fertilization success, but not on its mating success. Here, we do not find indications that $+/+$ and $+/t$ differ in their mating success (i.e. they did not differ in the total number of reproductive events, that is, copulations that resulted in offspring). Thus, several lines of evidence suggest that polyandry and sperm competition play a prominent role in t suppression, but the influence of additional factors can not be ruled out categorically. Customized computer simulations that make use of the known sperm competition parameters from our laboratory experiments may provide additional insight with regard to this question.

Overall, we find little evidence that female house mice exercise control over remating rate. Genetic polyandry rates seem largely determined by extrinsic environmental factors such as the availability of mating partners. Intrinsic factors such as individual identity or heritable genetic variation seem to play little role in trait expression. Throughout the sexual selection literature, monandrous behaviour is typically regarded as the default state, with polyandry requiring special explanation if observed. Kokko and Mappes (2013) have pointed out that the reverse could be the case, and that it may in fact be surprising if a biological organism, male or female, does not accept a mating when presented with the opportunity. Our results presented here are certainly in line with this position. At the same time, our study highlights that polyandry —irrespective of whether it is a the default state or an evolved property— can have fascinating repercussions on selection and trait evolution in males.

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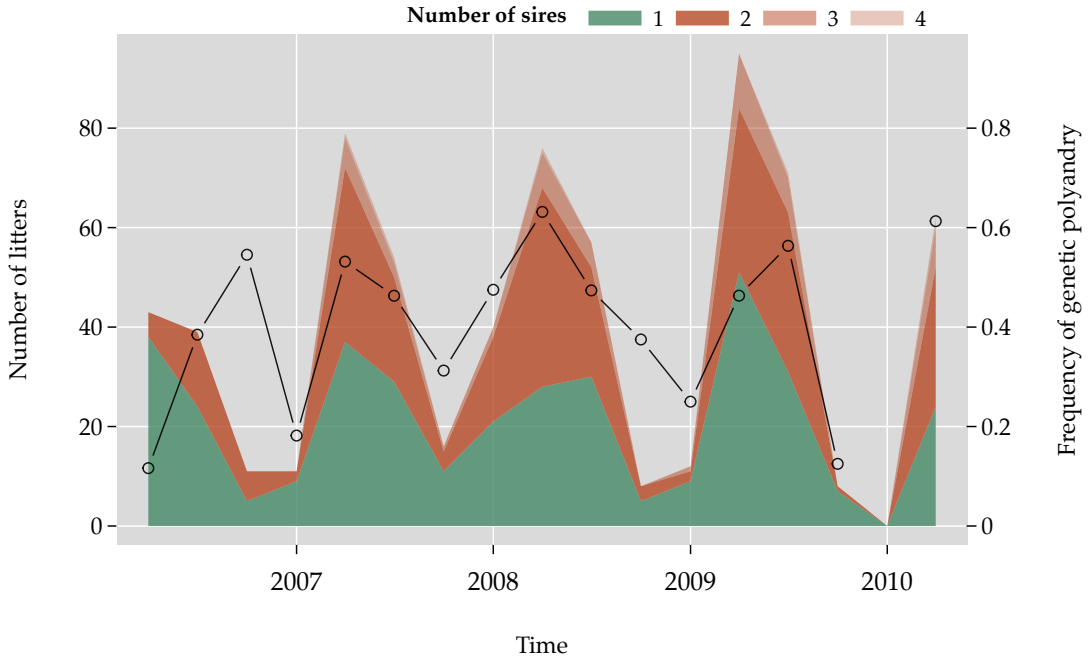

Figure S6.1. The number of litters (of size greater than one) born in quarterly year time intervals during the observation period. The number of monogamous litters (sired by one male only) are shown in green. The number of polyandrous litters (sired by two to four males, as indicated by the colour shading) are shown in red. The average genetic polyandry rates per quarter are shown as a black dotted line.

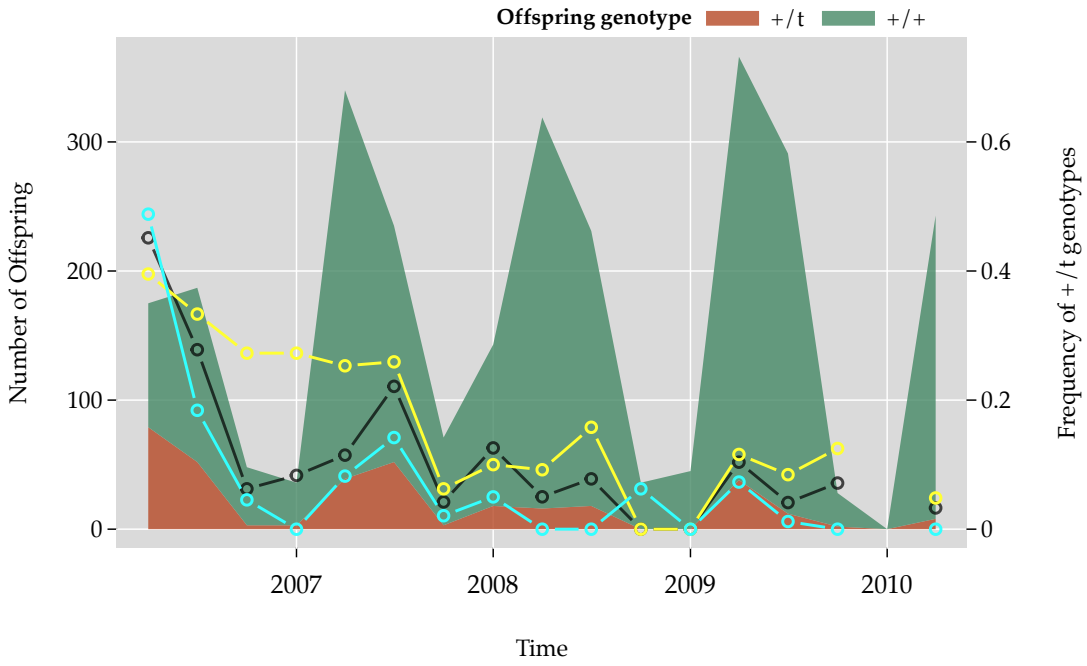

Figure S6.2. The *t* haplotype frequency in the population decreased during the observation period, as indicated by three different measures of +/ *t* genotype frequency (in quarterly year time intervals). The absolute number of +/*t* and +/+ individuals among new-born pups are shown in red and green, respectively. The black line shows +/*t* genotype frequency among pups. The yellow line depicts +/*t* genotype frequency among the females that were reproductively active during the given three-month period. The blue line indicates the +/*t* genotype frequency among successfully reproducing fathers. Note that *t* frequency among mothers are typically higher than the frequency among fathers, particularly in 2006/2007.

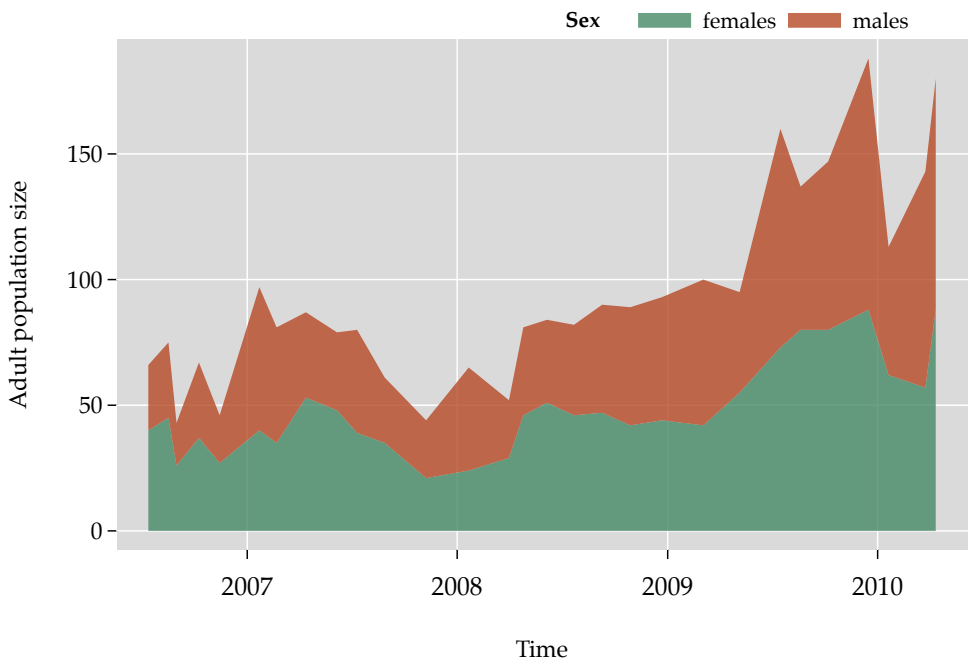

Figure S6.3. Adult male and female population size as registered during population-monitoring events during the observation period. Population density varied considerably, and overall increased in the 4.5 year observation period.

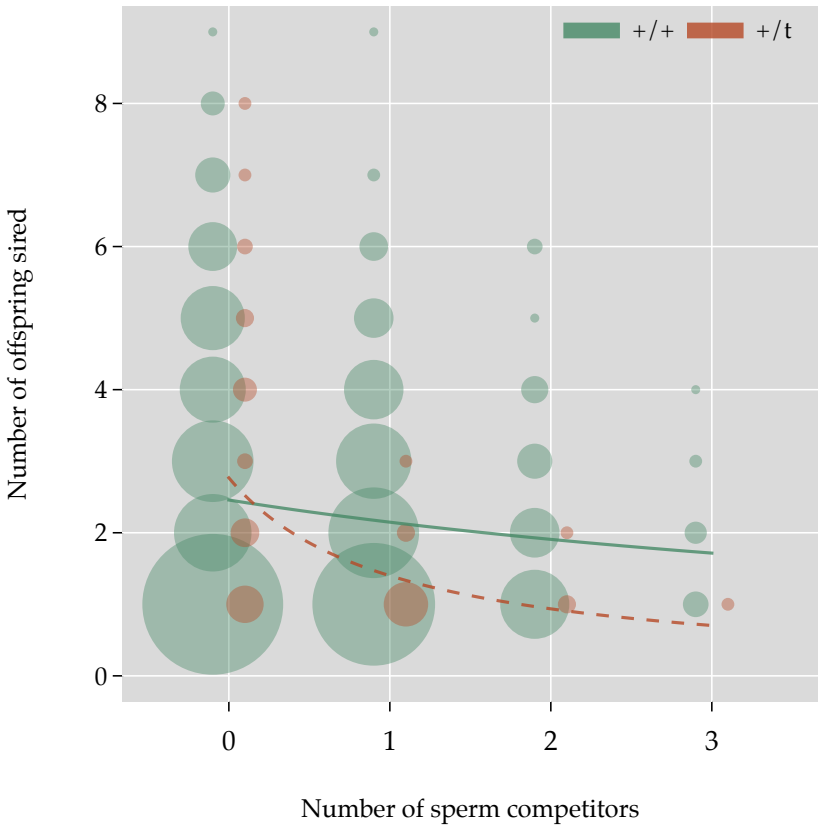

Figure S6.4. $+/t$ males are particularly compromised in sperm competition, as indicated by the interaction between sperm competition intensity (measured as the number of sperm competitors) and t genotype (red: $+/t$ males, green: $+/+$ males) on the number of offspring sired in *a single litter*. Solid and dotted lines show GLMM model predictions for $+/+$ and $+/t$ males, respectively.

Chapter 7

Synthesis

The aim of this thesis was to analyse the joint evolution of gene drive and female mating behaviour, guided by two research questions that are each related to an evolutionary paradox.

1. How does female mating behaviour affect the frequency dynamics of a drive gene (related to the low *t* frequency paradox)?

2. How does the presence of a drive gene affect the evolution of female mating behaviour (related to the lek paradox)?

Chapters 2 through 6 addressed the two questions from various angles, ranging from theoretical modelling to laboratory experiments and data analysis in the *t* haplotype system of house mice. In this synthesis, I will discuss the main findings with regard to questions 1 and 2. First, I will briefly outline the most important theoretical findings and their dependence on key parameters. Second, I shall summarize the empirical support for these theoretical ideas, as provided in this thesis and beyond. Third, I will discuss a few open questions and possible future directions.

7.1 Explaining Drive Frequencies (Question 1)

Throughout the thesis, and in chapters 3 and 4 in particular, we have attempted to assess the effect of female mating behaviour on the frequency dynamics of drive genes. In the conceptual framework (see General introduction, Figure 2), we asked how selection at the drive locus (drive axis) is affected by a *given* female mating behavior (suppressor axis). Two types of female mating behaviour have been predominantly discussed here (and elsewhere): precopulatory mate choice or polyandry and sperm competition. Note that answers to question 1 do not require that female mating behaviour has evolved *in response* to the presence of the driver (question 2). Hence, female mating behaviour, polyandry in particular, may be present for reasons that are independent of the driver (see below).

Theoretical Findings

Theoretical models as presented in this thesis (Chapter 2 and 4) as well as in earlier work (Haig and Bergstrom, 1995; Manser et al., 2011) suggest that female mating

behaviour has a strong potential to alter drive frequency dynamics. The theoretical predictions as to how mate choice and polyandry *qualitatively* affect drive frequency are intuitive (and almost trivial). t frequency equilibria will deviate from the standard prediction of $p_t = 0.33$ based on drive and homozygote lethality alone (Bruck, 1957), if females avoid t fertilisation either through precopulatory preferences or if females are polyandrous and the drive gene affects a male's sperm competitiveness. As pointed out in the general introduction, the main use of theoretical modelling with regard to question 1 is the development of specific *quantitative* predictions of drive dynamics that can subsequently be tested against observed frequencies (chapter 4). In addition to the two parameters that are already part of the standard model (*drive parameter d* and *homozygote lethality*), frequency predictions will depend on the following key parameters. *Polyandry parameter n* describes the size of the sample of males a female is mating with during a single reproductive event (Chapter 4). Any form of precopulatory choice will alter the *mean* frequency of drive-carrying males in a female's mate sample. In chapter 2, we have described this precopulatory mating preference strength with *preference parameter a*. Polyandry (if $n > 1$), on the other hand, will not modify the *mean*, but the *variance* frequency of drive males in a female mate sample. The more males a female is mating with, the *smaller* the sampling variation in her set of mating partners, the larger the role of postcopulatory sperm competition (chapter 4). We described the relative sperm competitiveness of a drive male relative its wild-type rivals by *sperm competitiveness parameter c* (chapter 4). In mini-chapter 5, we have further demonstrated that the efficiency of polyandry to reduce drive frequency will also depend on the intensity of viability selection acting on +/ heterozygote males and females (denoted with *selection coefficients parameters* s_f, s_m). Overall, both pre- and postcopulatory processes will effectively result in a *systematic deviation from random fertilisation.*

Empirical Support from the *t* Haplotype System

Systematic fertilization bias As pointed out above, both pre- and postcopulatory female drive avoidance strategies are expected to result in a systematic fertilization bias. We have provided ample empirical support that t haplotype carrying males do not manage to fertilize as many eggs as predicted by the standard model. In chapter 3, we specifically tested female mating behaviour in a controlled laboratory setting and found that $+/t$ managed to fertilize only few eggs under these conditions. Data from

the wild population also suggests that fertilization significantly deviates from the expectations based on random fertilization (chapter 6, Lindholm et al. (2013)). Let us now summarize, whether the observed bias is the result of pre- and/or postcopulatory mechanisms.

Drive males are poor sperm competitors [Parameter c] We have provided strong evidence from mice of different origin (Switzerland and Australia) that polyandry and subsequent sperm competition are a viable explanation for the observed fertilization bias. The mate choice experiment in chapter 3, that has principally allowed for both pre- and postcopulatory avoidance, strongly suggested an important role of sperm competition. In chapter 4, we provided direct evidence that $+/t$ males originating from Australian mouse populations are heavily compromised in their sperm competitive ability. A similar $+/t$ male disadvantage have been shown in controlled laboratory experiments on our Swiss study population (Sutter and Lindholm, 2015). The analysis in chapter 6 suggest that this disadvantage is also present (and fitness relevant) under natural conditions. Robust estimates of sperm competitiveness parameter c have previously been missing in this system. The solid estimates provided here and by Sutter and Lindholm (2015) are certainly an important step forward in our understanding of t haplotype frequencies under natural conditions.

Female mice are polyandrous [Parameter n] Sperm competition parameter c is only relevant if female house mice do mate with several males ($n > 1$), thereby allowing sperm competition to occur. Here, we show that female house mice regularly mate with more than one male, both when in a controlled laboratory setting (chapter 3) as well as in the natural population (chapter 6). In the experimental setup of chapter 3, where females were free to visit two males, female mice regularly visited, inspected, and mated with both males at disposition. Accordingly, about 30% of litters had a mixed paternity. Chapter 6 suggests that the mating patterns found in the lab setting translate to natural populations, as a substantial fraction of litters were sired by more than one male. Hence, there is striking similarity between the estimates from the laboratory and natural conditions. There is also a striking similarity between the estimates provided in this thesis and polyandry rates of wild house mice reported elsewhere (Rolland et al., 2003; Dean et al., 2006; Firman and Simmons, 2008a).

Weak evidence for precopulatory mate choice [Parameter a] In the literature, the t haplotype system is usually mentioned as one of the rare cases in which females avoid drive males prior to mating (e.g. Wedell (2013)). In a series of experiments conducted on a North American house mouse population, $+/t$ females (and

sometimes +/+ females) were repeatedly shown to avoid +/*t* males based on an olfactory basis (reviewed in Lenington (1991)). Despite several attempts to replicate these findings, we find little evidence for such precopulatory choice in our population of Swiss house mice. The experiments of chapter 3 did not reveal clear visiting patterns and paternity outcomes were largely compatible with scenarios that assume postcopulatory process only. Under natural conditions (chapter 6), we did not find that +/*t* and +/+ males significantly differed in the overall number of reproductive events. However, it is important to note that methods used here to infer preference parameter *a* were indirect. That is, mating success was not observed directly, but deduced from a male's paternity success. As a result, our inferences about mating patterns always are conditional on underlying assumptions about the postcopulatory mechanisms that determine a male's mating success (such as order effects, skew etc.).

Is the *t* Frequency Paradox Resolved?

Are the parameter values as measured here sufficient to explain the observed *t* haplotype frequency dynamics? In other words, can female mating behaviour, polyandry and sperm competition in particular, resolve the the low *t* frequency paradox for good? First of all, we have provided additional evidence for the fact that observed *t* frequency dynamics, both under natural (chapter 6) and laboratory (chapter 4) conditions are lower that expected by the standard prediction (Bruck, 1957). The observed *t* frequency patterns observed here fit well into the picture reported in the literature (e.g. Ardlie and Silver (1996); Burt and Trivers (2006)) and show that the *t* frequency paradox is a real problem.

In chapter 4, we have directly compared updated model predictions that include sperm competition effects to observed frequency dynamics in the selection lines. Surprisingly, we ended up with the reverse problem: even though observed *t* frequencies were significantly reduced in polyandrous lines, the parameterized model predicted frequencies that were substantially lower than that. We have no good explanation for this discrepancy at the moment, and the causes of this 'inverse paradox' certainly deserve further investigation. In chapter 6, we have reported a substantial decrease in *t* frequency during the observed period. Explicit model predictions for the *t* frequency dynamics in the study population have been developed during my MSc project (Manser et al., 2011). The new data provided in chapter 6, together with the recently measured sperm competition parameters (Sutter and Lindholm, 2015), suggest that

sperm competitive effects may be sufficient to drive the t haplotype to the brink of extinction.

The sperm competitive disadvantage of $+/t$ males is dramatic and robust. Moreover, house mice are highly polyandrous. Note that polyandry rates are typically increased with population density (as reported here in chapter 6). As a result, a polyandry model can also explain the previously reported negative relationship between observed t frequencies and population size (Ardlie and Silver, 1998). Overall, the addition of sperm competitive effects to the standard model is certainly a leap forward in our understanding of t frequency dynamics. This does evidently not deny the potential importance of other evolutionary forces in the system.

Open Questions and Future Directions

Geographic and phylogenetic comparisons The evidence in favour of the polyandry hypothesis presented here is based on observations from two single populations. Yet the comparison of different populations within the same species, as well as among species have proved a powerful tool to test evolutionary hypotheses. For example, Price et al. (2014) have reported a latitudinal cline in polyandry rates in *Drosophila pseudoobscura*. They were able to show that the frequency of a sex-ratio driver occurred at lower frequencies in populations with higher remating rates; compelling evidence for the role of polyandry in sperm competition. Studies that relate multiple paternity rates to t frequency across populations of the *Mus musculus domesticus* cluster, or between different mouse subspecies, would certainly further corroborate the role of sperm competition in the t haplotype context.

Selection on male sperm traits We find that the two male genotypes strongly differ in their sperm competitive ability. This circumstance may not only affect trait selection in females (as discussed under question 2), but also in males. For example, as a result of the asymmetry in sperm quality, we may expect $+/t$ males to allocate more resources into sperm production, gaining more matings or acquiring social dominance (or mate guarding) to avoid sperm competition. Engqvist (2012) has explored the possibility of differential ejaculate investment using a game-theoretical framework. The model has demonstrated that the optimal ejaculate investment can indeed differ if males systematically differ in sperm quality. Typically, it is the less fertile males that should invest more. As discussed in chapter 4, such compensatory male investment by $+/t$ males may potentially explain the unexpectedly high t frequencies in polyandrous

lines. However, Engqvist's model has assumed that the frequency of male genotypes in a population is fixed. Because less fertile males can never fully compensate for their sperm competitive disadvantage, it is not clear whether his conclusions would hold in circumstances where the frequency of the male types can evolve (as was the case here). Note that the drive effect will help to maintain $+/t$ males even if they cannot fully compensate for their low sperm quality *individually*. Furthermore, the model neglects the co-evolutionary feedback of male compensatory investment on female behaviour. Such a feedback on females seems extremely likely, as any $+/t$ male strategy to increase his fertilisation success will clearly conflict with the evolutionary interests of the females. In our Swiss mouse population, Sutter and Lindholm (2015) have measured the size of several male reproductive organs, and have found no indication for differential investment between $+/t$ and $+/+$ males. An earlier study that has compared sperm quantity at three stages before and after ejaculation did also detect no effect of male t genotype (Silver and Olds-Clarke, 1984). The evolution of male mating strategy in a scenario where male genotypes as well as female behaviour are free to evolve certainly deserves further theoretical investigation. Moreover, the possibility of behavioural $+/t$ male compensation by means of increased aggression/dominance (see next section) or an increased mating acquisition also looks worth exploring in a theoretical framework.

A role for precopulatory male-male competition or cryptic female choice? The sexual selection processes that have mainly been considered as drive suppressors, in this thesis and beyond, are either intersexual/precopulatory (female mate choice) or intrasexual/postcopulatory (sperm competition). However, sexual selection may also create a fertilisation bias through intrasexual male-male competition before mating, or postcopulatory female choice (cryptic female choice). Indeed, previous work has suggested a relationship between the t haplotype and male dominance. However, empirical evidence is contradictory at best: while some studies have found $+/t$ males to be dominant in staged male-male encounters (Lenington, 1991; Lenington et al., 1996), another has suggested the opposite pattern (Carroll et al., 2004). Moreover, Lindholm et al. (2013) has found that t haplotype transmission rates (drive) is about 10% lower in monogamous crosses between two $+/t$ heterozygotes compared to a monogamous cross between a $+/+$ female and a $+/t$ male. Cryptic female choice (egg-sperm interaction) is a viable explanation for this pattern. However, the measured effect is probably too small to have strong repercussions on population drive frequency.

By-product or evolved property? At the beginning of this section, we have stated that solid answers to question 1 do not require female mating behaviour, polyandry

in particular, to have evolved *as a consequence* of the presence of the driver. Accordingly, the model provided in chapter 4 (and in Manser et al. (2011) have regarded female polyandry rate as a fixed rather than an evolvable quantity. In such a modelling approach, it was irrelevant whether polyandry has evolved as a drive suppressor or whether it is present for reasons unrelated to drive. However, the models provided in chapters 1 and 2 suggest that this conclusion may be premature. Obviously, the frequency of polyandry in a population will have a strong impact on the extent of *t* drive suppression in a population. In a case where selection on polyandry *solely* depends on its effects on drive suppression, as modelled in chapters 2, we can not ignore the dynamic co-evolutionary feedback between drive and polyandry. Accordingly, our models have suggested that both polyandry and female mate choice —due to the lek problem— can never *fully* remove the driver from the population. This is because costly female drive suppression will typically be subjected to negative selection as soon as the driver is lost (due to a lack of variation in male genetic quality). Once the intensity against the driver is decreased, it can spread once more. This intricate effect potentially explains why observed *t* frequencies in natural populations are typically low, but stable Ardlie (1998). However, note that this argument is based on the assumption that female drive avoidance is in fact 'drive-driven'. The evidence for this possibility is summarized in the next section.

7.2 Explaining Female Mating Behaviour (Question 2)

In this second question, we have investigated whether the presence of a drive gene affects the evolution of female mating behaviour. Note that, while the reverse was not necessarily the case (see last paragraph), question 2 crucially depends on our findings of question 1. This is because the *avoidance* of drive male fertilization (as relevant in question 1) and female *benefits* related to polyandry or mate choice (as relevant in question 2) are flip sides of the same coin.

Theoretical Findings

The models provided in chapter 2 suggest that mating behaviour can readily evolve as female countermeasures against drive genes and their harmful effects. Importantly,

this is also the case if such mating behaviour is associated with direct fitness costs. For an ample range of the parameter space considered, female drive suppression is both beneficial and evolutionarily stable. Avoiding drive males is *beneficial* to females because it helps females avoid drive related fitness costs in their offspring. Avoiding drive males is *evolutionarily stable* because, thanks to drive, drive males remain in the population despite natural and sexual selection against them. As a result, variation in male genetic quality is maintained *at equilibrium*. Thanks to this circumstance, the lek paradox is largely avoided.

Our modelling efforts have highlighted that, even in a system where the lek paradox only plays a minor role, successful evolution of polyandry or mate choice is predicated on several key parameters. As expected, we find that if the fitness costs associated with drive avoidance (described by *parameter* c_p) are too large, avoidance will not be positively selected. The relationship between preference strength (*parameter* a) and preference evolution is intriguing. If the preference is weak, drive avoidance remains inefficient, thereby rendering positive selection on preference unlikely. If preferences are strong, drive avoidance evolution is limited because it is, in some sense, *too efficient*. In this case, benefits of drive avoidance become small because the strong preferences push drive frequency to the brink of extinction, thereby eroding possible benefits associated with choice. Note that in the polyandry scenario, parameter a is equivalent to the combined action of *mating partner number* n and *sperm competitiveness cost* c. In the mate choice scenario, we further find that the presence of an indicator trait of a male's drive status is an essential prerequisite for preference evolution (as measured by *recombination parameter* r). Finally, in modelling female mating behaviour as a qualitative trait determined by a single locus, we implicitely assume heritable genetic variation in the female trait of interest (measured by *parameter* h^2). In the absence of such additive genetic variation, the trait cannot evolve even if it is under positive or negative selection.

Empirical Support from the *t* Haplotype System

Mixed evidence for female fitness benefits related to drive avoidance [Parameters a, n, c, and d] As mentioned at the beginning of this section, we expected a direct relationship between the parameters that determine the *benefits* of female drive avoidance and the parameters that affect drive avoidance (as discussed under Question 1). In chapter 3, we showed that drive avoidance indeed increases a

$+/t$ female's reproductive success (the evolutionary implications of possible differences between $+/t$ and $+/+$ females are discussed below). Sutter and Lindholm (2015) have measured the litter size benefits of multiple mating by directly counting t/t lethal embryos in the female reproductive tract. This more precise method has confirmed that polyandry can greatly help $+/t$ females to avoid litter losses. In spite of the strong experimental evidence for genetic benefits of polyandry, we found few signs of selection on polyandry in a natural context. In fact, there was no evidence for selection on t genotype, polyandry nor their interaction in females. Surprisingly, we also saw no litter size benefits related to polyandry in the Australian mice (chapter 4). We have hypothesised that the timing of the lethal effect during embryo development may play an important role in this case. If t/t embryos perish before the embryos are implanted, they may be replaced by viable embryos. The possibility of such a compensation of inviable embryos before implantation in the uterus has so far been neglected in the t haplotype literature. However, if real, the compensation of litter losses will have major adaptive repercussions, because the evolution of costly drive avoidance seems unlikely under these circumstances. Consequently, the hypothesis certainly deserves further investigation.

Costs of female drive avoidance are largely unknown [Parameter c_p] Experimentally measuring the costs associated with polyandry is extremely difficult, and we have not attempted to explicitly quantify costs in this thesis. Given absence of selection on polyandry in females, one might be tempted to argue that there are low costs of polyandry in the study population (chapter 6). However, the absence of selection does not imply the absence of trait costs, but only that costs and potential benefits are in balance. Flat selection gradients may thus imply that polyandry frequency in our population is at a selective equilibrium. However, in light of the asymmetry between $+/+$ and $+/t$ females in terms of benefits, the idea that polyandry-related costs are exactly equal and opposite in *both* genotypes appears rather unlikely (and would further imply *unequal* polyandry costs in the two genotypes). Another possibility is that polyandry costs do not show in the fitness measure used here. Chapter 6 has analysed the effect of promiscuous behaviour on overall reproduction. The costs related to polyandry (such as increased exposure to disease), however, may mainly manifest themselves in female survival rates.

Little evidence for heritability of polyandry [Parameter h^2] In chapter 6, we have quantified, for the first time in house mice, the amount of additive genetic variance for polyandry in a context that is relevant for selection (i.e. in a wild population).

We have found little evidence that polyandry is heritable in our study population.

In summary, we can say that based on theory, we expect females to readily evolve strategies to avoid drive fertilisation (polyandry in particular, see next section). However, we find little evidence that this was the case in the specific contexts measured here.

Why Polyandry Is a More Likely Drive Suppressor than Mate Choice

As summarized under question 1, the evidence provided throughout this thesis highlighted the importance of polyandry and sperm competition on *t* frequency dynamics. On the other hand, we find little to suggest the presence of female drive avoidance prior to mating. This conclusion fits well into the larger picture. A number of studies, conducted on drive systems across several taxa, have highlighted the importance of sperm competition on drive dynamics (reviewed in Wedell (2013)). Evidence for precopulatory mate choice, on the other hand, is scarce (Wedell, 2013). Drive suppression by sexual selection can either be considered an evolved reaction to the presence of driver or a by-product of other adaptive or non-adaptive processes (see section above). In both scenarios, there are good reasons why polyandry is more likely to suppress drive than precopulatory choice.

The model presented in chapter 2 has demonstrated that precopulatory choice is less likely to evolve as a drive suppression mechanism in a case where female mating behaviour evolves *in reaction* to the presence of drive. The model highlights that the presence of a reliable male signal, indicating the presence/absence of the drive gene, is an indispensable precondition for the evolution of precopulatory drive avoidance. Accordingly, already the smallest degree of recombination (*parameter r*) between the male signal and the driver resulted in the disappearance of female choice. The necessary presence of a recognisable drive signal may not render precopulatory choice impossible in all cases, but it is certainly a very restrictive prerequisite. In the case of polyandry, on the other hand, female do not need an indicator of male drive status. This is because the key process that helps females avoid drive fertilization in this case is sperm competition *between* males (intrasexual competition). Such male-male competition *does not require* female interaction.

For related reasons, polyandry also seems more likely in a scenario where female drive suppression is a by-product of an adaptive or non-adaptive process that is *in-*

dependent of drive. Polyandry does not require interactive effects between females and *specific* male genetic regions. Polyandry is, in this sense, a very broad and unspecific process. All genes with an impact on male sperm traits will be equally affected by sperm competition, irrespective of their *specific* position in the genome. Most proposed precopulatory mate choice processes, on the other hand, seem to target rather specific genomic regions (e.g. MHC complex). Thus, the probability that the driver will accidentally find itself in such a specific region appears small. Overall, the odds of observing polyandry and sperm competition rather than precopulatory choice as a drive suppression mechanism appear high, and the empirical observations seem to support this conjecture.

Open Questions and Future Directions

Experimental evolution for increased polyandry rates As discussed above, we have found little evidence for selection on female mating behaviour in response to the presence of gene drive under the specific circumstances measured here. However, the female fitness effects under laboratory conditions (chapter 3) seem too dramatic to abandon the idea of drive-driven polyandry altogether. In chapter 4, we analysed selection at the t haplotype as a function of female mating behaviour, holding the latter constant (question 2). The opposite experiment may be equally fascinating: one could create different selective environments by manipulating the *frequency* of $+/t$ males among different selection lines (holding male drive frequency within a selection line constant). Under such circumstances, we expect selection for increased polyandry rates in lines that contain a high proportion of $+/t$ males. The one study that has so far directly addressed this question in a sex ratio drive system of *Drosophila pseudoobscura* Price et al. (2008a), has indeed found elevated remating rates in lines where drive frequencies were high.

Good genes vs compatible genes: do we expect differences in mating behaviour between +/+ and +/t females? An important issue that has received little attention so far is the question whether we would expect $+/t$ and $+/+$ to differ in their levels of drive avoidance (pre- or postcopulatory). In the general introduction, we have presented the idea that females should choose males with highest additive genetic quality ('good genes' or 'good sperm' hypothesis). However, variation individual genetic quality may also stem from non-additive genetic variance such as dominance or epistasis. Importantly, if such non-additive effects are important, a female's best choice

may not only depend on the genotype of the male partner, but on the *interaction* between her own and the partner's genotype (Trivers, 1972). Thus, under the concept of mate choice for genetic compatibility, a good choice for one female may be a bad one for another. Note that under such a scenario, the lek problem may be avoided.

Drive systems have been put forward as strong candidates for compatibility mate choice (Tregenza and Wedell, 2000; Zeh and Zeh, 1996). Indeed, the organismal fitness effect of the t haplotype is highly non-additive, as only t/t homozygotes suffer from the lethal mutations (dominance). As a result, we have shown that *benefits* of drive avoidance are dramatically different between $+/+$ and $+/t$ genotypes (chapter 3). Yet, it is unclear whether we would expect $+/t$ and $+/+$ female to differ in their polyandry or mate avoidance rates. Firstly, an asymmetry in benefits does *not* imply opposing selection. Even though fitness payoffs are not identical, one can make a good case that drive avoidance is beneficial to *both* female genotypes. As pointed out in chapters 1 and 3, avoiding t fertilisation would help $+/+$ to avoid producing sons with impaired sperm competitive ability and/or low attractiveness to females. Secondly, opposing selection does not imply divergent evolution. Thus, even in a case where drive avoidance is selectively favoured in $+/t$ females while disfavoured in $+/+$ females (opposing selection), it is unclear how such systematic preference / polyandry differences between $+/t$ and $+/+$ females could be maintained mechanistically. For example, one might imagine that exclusive drive avoidance by $+/t$ females is 'genetically implemented' with a choice allele that is physically linked to the t haplotype Lenington (1991). Yet it is difficult to see how such a t haplotype variant carrying its own suppressor could be competitive against rival t haplotypes. In light of mini-chapter 5, it seems unlikely that the selective benefit of such a t variant in the female function would outweigh the cost of reduced $+/t$ male success in the male function. If anything, drive avoidance will result in an association between the suppressor allele and the non-driving wildtype allele (because females that carry the choice allele will produce a smaller proportion of drive carrying individuals, see chapter 2). Additional theoretical models are clearly necessary to assess whether a genotype-specific divergence in female mating behaviour is feasible under certain circumstances. In any case, we did not find strong evidence for systematic differences in drive avoidance between $+/t$ and $+/+$ in the studies provided here (chapters 3 and 6).

7.3 Concluding Remarks

In summary, the present work provides compelling theoretical and empirical evidence that polyandry and sperm competition strongly impact the frequency dynamics of t haplotypes in this system. Drive carrying $+/t$ males are poor sperm competitors and female mice are highly polyandrous. The addition of these two effects to the standard model greatly improves t frequency predictions, and goes a long way in explaining the low t frequencies typically observed under natural conditions (t frequency paradox). On theoretical grounds, we show that the presence of gene drive greatly facilitates the evolution of female strategies to avoid drive, thereby avoiding the lek paradox. While we find signs of such positive selection on female drive avoidance strategies under controlled laboratory conditions, we do not see evidence that drive impacted mating behaviour evolution in a natural context.

Overall, this thesis demonstrates that uncovering the hidden action of drive systems is a worthwhile endeavour. It highlights that the intricacies of multilevel conflict and its resolutions can deepen our general understanding of biological systems, in this case mating system evolution. Importantly, drive genes provide us with insights that could not be had under the notion of organisms as the sole fitness maximising agents. Finally, drive systems are an important ingredient to our *conceptual* understanding of the evolutionary process. As discussed at the outset, multilevel selection and related concepts such as kin selection and group selection have evoked heated scientific and philosophical debates over the years (Okasha, 2006). Drive systems give us the unique opportunity to complement these conceptual and theoretical discussions with observations from the real world. In a recent article, Steven A. Frank has concluded with the following words (Frank, 2011).

> There is a large philosophical literature on the meaning of *individuality* and of *units of selection* in relation to *levels of selection* (Sober and Wilson, 1994; Okasha, 2006). One can certainly learn from studying that philosophical literature. However, I have found it more instructive to analyse a wide range of interesting biological problems, to discover in practice what is actually needed to understand those problems, and to learn what general concepts link the different problems within a common conceptual basis (cf. Michod (1997, 2006). Philosophical induction from numerous evolutionary deductions.

I hope that the work presented here has added a few of those *evolutionary deductions* from a fascinating biological system.

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